

CLAIMS

1. 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; and/or
- 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; and/or
- 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, and/or
- 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/or

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.

2. The modified IL-2 polypeptide of claim 1, which comprises a substitution with lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, alanine, tryptophan, isoleucine, phenylalanine, or tyrosine 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.

3. The modified IL-2 polypeptide of claim 1 or 2, which:

- a) comprises a substitution with a natural amino acid 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 is configured to be

conjugated to a water-soluble polymer, a lipid, a protein, or a peptide at the 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/or

b) comprises a substitution with a natural amino acid 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 is configured to be conjugated to a water-soluble polymer, a lipid, a protein, or a peptide at the N terminal and/or C terminal of the polypeptide.

4. The modified IL-2 polypeptide of claim 3, which:

a) comprises a substitution with lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, alanine, tryptophan, isoleucine, phenylalanine, or tyrosine 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/or

b) comprises a substitution with lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, alanine, tryptophan, isoleucine, phenylalanine, or tyrosine at a position selected from the group consisting of N30, Y31, N33, P34, K35, R38, T41, K43, K48, K49, K64, P65, N71, Q74, K76 and a combination thereof.

5. The modified IL-2 polypeptide of claim 4, which:

a) comprises a substitution with cysteine at a position selected from the group consisting of N29, N30, Y31, N33, P34, K35, R38, T41, K43, K48, K49, K64, P65, N71, Q74, K76 and a combination thereof;

b) comprises a substitution with cysteine at a position selected from the group consisting of N29, Y31, K35, P65, N71, Q74 and a combination thereof;

c) comprises a substitution with any amino acid at a position of Y31, N29 or a combination thereof;

d) comprises a substitution with cysteine, serine or alanine at a position of Y31, N29 or a combination thereof;

e) comprises a substitution with cysteine at a position of Y31;

f) comprises a substitution with cysteine at a position of N29; and/or

g) comprises a substitution with cysteine at a position of P65.

6. The modified IL-2 polypeptide of any of claims 3-5, which further comprises 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.

7. The modified IL-2 polypeptide of claim 6, which further comprises a substitution with a natural amino acid at a position within IL-2R α interaction region.

8. The modified IL-2 polypeptide of claim 7, which comprises a substitution with a natural amino acid at a position selected from the group consisting of R38, F42, Y45, E62, P65 and a combination thereof.

9. The modified IL-2 polypeptide of claim 8, which comprises a substitution with lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, alanine,

tryptophan, isoleucine, phenylalanine, or tyrosine at a position selected from the group consisting of R38, F42, Y45, E62, P65 and a combination thereof.

10. The modified IL-2 polypeptide of claim 6 or 9, which:
 - a) comprises a substitution with cysteine at a position selected from the group consisting of R38, F42, Y45, E62, P65 and a combination thereof;
 - b) comprises a substitution with alanine, lysine or serine at a position of F42;
 - c) comprises a substitution with alanine at a position of F42;
 - d) comprises a substitution with serine at a position of F42;
 - e) comprises a substitution with lysine at a position of F42;
 - f) comprises a substitution with alanine, histidine or serine at a position of Y45;
 - g) comprises a substitution with alanine at a position of Y45;
 - h) comprises a substitution with histidine at a position of Y45;
 - i) comprises a substitution with alanine, aspartic acid or serine at a position of R38;
 - j) comprises a substitution with aspartic acid at a position of R38;
 - k) comprises a substitution with alanine at a position of P65;
 - l) comprises a substitution with serine at a position of P65;
 - m) comprises a substitution with alanine at a position of E62; and/or
 - n) comprises a substitution with lysine at a position of F42, a substitution with cysteine at position of Y31, or a combination thereof.
11. The modified IL-2 polypeptide of claim 6, which further comprises a substitution with a natural amino acid at a position within IL-2R β interaction region.
12. The modified IL-2 polypeptide of claim 11, which comprises a substitution with a natural amino acid at a position selected from the group consisting of Q13, L19, R81, L85, S87, V91, I92, V93 and a combination thereof.
13. The modified IL-2 polypeptide of claim 12, which comprises a substitution with lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, alanine, tryptophan, isoleucine, phenylalanine, or tyrosine at a position selected from the group consisting of Q13, L19, R81, L85, S87, V91, I92, V93 and a combination thereof.
14. The modified IL-2 polypeptide of claim 13, which comprises a substitution with cysteine at a position selected from the group consisting of Q13, L19, R81, L85, S87, V91, I92, V93 and a combination thereof.
15. The modified IL-2 polypeptide of any of claims 6-14, which further comprises:
 - a) a substitution with a natural amino acid at a position within IL-2R α interaction region and a substitution with a natural amino acid at a position within IL-2R β interaction region;
 - b) a substitution with a natural amino acid at a position within IL-2R α interaction region and a substitution with a natural amino acid at a position within IL-2R γ interaction region; or
 - c) a substitution with a natural amino acid at a position within IL-2R α interaction region, a substitution with a natural amino acid at a position within IL-2R β interaction region and a substitution with a natural amino acid at a position within IL-2R γ interaction region.

16. The modified IL-2 polypeptide of any of claims 1-15, which has reduced binding to an 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.
17. The modified IL-2 polypeptide of claim 16, wherein its binding affinity to an IL-2R α is decreased from about 10% to about 100%, or is decreased from about 1 fold to about 100,000 fold or more.
18. The modified IL-2 polypeptide of claim 16, which has no detectable binding to an IL-2R α .
19. The modified IL-2 polypeptide of any of claims 1-18, which 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.
20. The modified IL-2 polypeptide of claim 19, wherein a ratio between its signaling potency to IL-2R $\alpha\beta\gamma$ and the signaling potency to IL-2R $\alpha\beta\gamma$ of the comparable IL-2 polypeptide comprising an amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 without the substitution is from about 1/2 to about 1/100,000.
21. The modified IL-2 polypeptide of claim 19, which has no detectable receptor signaling potency to IL-2R $\alpha\beta\gamma$.
22. The modified IL-2 polypeptide of any of claims 1-21, which has reduced binding to an 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 and 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.
23. The modified IL-2 polypeptide of claim 22, which has no detectable binding to an IL-2R α and has no detectable receptor signaling potency to IL-2R $\alpha\beta\gamma$.
24. The modified IL-2 polypeptide of any of claims 1-23, which retains substantial or has higher binding level to an interleukin 2 receptor β (IL-2R β) or an interleukin 2 receptor γ (IL-2R γ) 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 retains substantial or has higher 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.
25. The modified IL-2 polypeptide of claim 24, which retains substantial or has higher binding level to an IL-2R β or an IL-2R γ 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.
26. The modified IL-2 polypeptide of claim 24, which retains substantial or has higher 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.

27. The modified IL-2 polypeptide of claim 24, which retains substantial or has higher binding level to an IL-2R β or an IL-2R γ 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 retains substantial or has higher 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.

28. The modified IL-2 polypeptide of claim 1-27, which 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.

29. The modified IL-2 polypeptide of any of claims 1-28, which has a N terminal deletion, *e.g.*, a N terminal deletion of amino acid residues 1-30, and/or a C terminal deletion, *e.g.*, a C terminal deletion of amino acid residues 114-134.

30. The modified IL-2 polypeptide of any of claims 1-29, which has a N terminal deletion and a C terminal deletion.

31. The modified IL-2 polypeptide of any of claims 1-30, which is a part of a fusion polypeptide, *e.g.*, a recombinant fusion protein, that comprises the modified IL-2 polypeptide and an additional amino acid sequence.

32. The modified IL-2 polypeptide of claim 31, wherein the N terminus or the C terminus of the modified IL-2 polypeptide is fused to the additional amino acid sequence.

33. The modified IL-2 polypeptide of claim 32, wherein the additional amino acid sequence comprises an antibody sequence or a portion or a fragment thereof, *e.g.*, a Fc portion of an antibody.

34. The modified IL-2 polypeptide of any of claims 1-33, which is in an isolated form.

35. A polynucleotide, *e.g.*, DNA, RNA or viral vector, that encodes a modified IL-2 polypeptide of any of claims 1-34 and is configured to express said modified IL-2 polypeptide *in vitro* and/or *in vivo*.

36. A modified IL-2 polypeptide conjugate, which comprises a modified IL-2 polypeptide of any of claims 1-34 or 1-35 that is conjugated to a water-soluble polymer, a lipid, a polypeptide, *e.g.*, a protein, or a peptide.

37. The modified IL-2 polypeptide conjugate of claim 36, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide covalently.

38. The modified IL-2 polypeptide conjugate of claim 36, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide non-covalently.

39. The modified IL-2 polypeptide conjugate of any of claims 36-38, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted natural amino acid or 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.

40. The modified IL-2 polypeptide conjugate of claim 39, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted natural 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.

41. The modified IL-2 polypeptide conjugate of claim 39, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, alanine, tryptophan, isoleucine, phenylalanine, or tyrosine 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.

42. The modified IL-2 polypeptide conjugate of claim 39, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted cysteine 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.

43. The modified IL-2 polypeptide conjugate of claim 39, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted natural amino acid or unnatural amino acid 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.

44. The modified IL-2 polypeptide conjugate of claim 43, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted natural amino acid 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.

45. The modified IL-2 polypeptide conjugate of claim 44, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, alanine, tryptophan, isoleucine, phenylalanine, or tyrosine 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.

46. The modified IL-2 polypeptide conjugate of claim 45, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a substituted cysteine 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.

47. The modified IL-2 polypeptide conjugate of any of claims 36-46, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a single amino acid residue of the modified IL-2 polypeptide.

48. The modified IL-2 polypeptide conjugate of claim 47, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via:
i) the alpha amino group of the N-terminal amino acid residue of the modified IL-2 polypeptide;
ii) the epsilon amino group of a lysine amino acid residue of the modified IL-2 polypeptide; or
iii) an N-glycosylation site or O-glycosylation site of the modified IL-2 polypeptide.

49. The modified IL-2 polypeptide conjugate of any of claims 36-48, wherein the modified IL-2 polypeptide is covalently conjugated to a water-soluble polymer, a lipid, a protein, or a peptide through a linker.

50. The modified IL-2 polypeptide conjugate of any of claims 36-46, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via a single amino acid residue in a fusion polypeptide that comprises the modified IL-2 polypeptide and an additional amino acid sequence.

51. The modified IL-2 polypeptide conjugate of claim 50, wherein the single amino acid residue is located within the modified IL-2 polypeptide.

52. The modified IL-2 polypeptide conjugate of claim 50, wherein the single amino acid residue is located within the additional amino acid sequence.

53. The modified IL-2 polypeptide conjugate of any of claims 50-52, wherein the additional amino acid sequence comprises an antibody sequence or a portion or a fragment thereof.

54. The modified IL-2 polypeptide conjugate of claim 53, wherein the additional amino acid sequence comprises a Fc portion of an antibody.

55. The modified IL-2 polypeptide conjugate of any of claims 50-54, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer, a lipid, a protein, or a peptide via:
i) the alpha amino group of the N-terminal amino acid residue of the fusion polypeptide;
ii) the epsilon amino group of a lysine amino acid residue of the fusion polypeptide; or

iii) an N-glycosylation site or O-glycosylation site of the fusion polypeptide.

56. The modified IL-2 polypeptide conjugate of claim 55, wherein the fusion polypeptide is covalently conjugated to a water-soluble polymer, a lipid, a protein, or a peptide through a linker.

57. The modified IL-2 polypeptide conjugate of any of claims 36-56, wherein the modified IL-2 polypeptide is conjugated to a water-soluble polymer.

58. The modified IL-2 polypeptide conjugate of claim 47, 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.

59. The modified IL-2 polypeptide conjugate of claim 58, wherein the water-soluble polymer comprises a PEG molecule.

60. The modified IL-2 polypeptide conjugate of claim 59, wherein the PEG molecule is a linear PEG.

61. The modified IL-2 polypeptide conjugate of claim 59, wherein the PEG molecule is a branched PEG.

62. The modified IL-2 polypeptide conjugate of claim 61, wherein the branched PEG has about three to about ten PEG chains emanating from a central core group.

63. The modified IL-2 polypeptide conjugate of claim 61, wherein the branched PEG is a star PEG comprising from about 10 to about 100 PEG chains emanating from a central core group.

64. The modified IL-2 polypeptide conjugate of claim 61, wherein the branched PEG is a comb PEGs comprising multiple PEG chains grafted onto a polymer backbone.

65. The modified IL-2 polypeptide conjugate of any of claims 59-64, wherein the PEG molecule has a range of molecular weight from about 300 g/mol to about 10,000,000 g/mol.

66. The modified IL-2 polypeptide conjugate of any of claims 59-64, wherein the PEG molecule has an average molecular weight from about 5,000 Daltons to about 1,000,000 Daltons.

67. The modified IL-2 polypeptide conjugate of claim 66, wherein the PEG molecule has an average molecular weight of from about 20,000 Daltons to about 30,000 Daltons.

68. The modified IL-2 polypeptide conjugate of any of claims 59-67, wherein the PEG molecule is a monodisperse, uniform, or discrete PEG molecule.

69. The modified IL-2 polypeptide conjugate of claim 57, wherein the water-soluble polymer comprises a polysaccharide.
70. The modified IL-2 polypeptide conjugate of any of claims 36-56, wherein the modified IL-2 polypeptide is conjugated to a lipid.
71. The modified IL-2 polypeptide conjugate of claim 70, wherein the lipid comprises a fatty acid.
72. The modified IL-2 polypeptide conjugate of any of claims 36-56, wherein the modified IL-2 polypeptide is conjugated to a protein.
73. The modified IL-2 polypeptide conjugate of claim 72, wherein the protein comprises an antibody or a binding fragment thereof.
74. The modified IL-2 polypeptide conjugate of claim 73, wherein the antibody or a binding fragment thereof comprises an Fc portion of an antibody.
75. The modified IL-2 polypeptide conjugate of any of claims 36-74, wherein a water-soluble polymer, a lipid, a protein, or a peptide is indirectly bound to the substituted natural amino acid or unnatural amino acid of the modified IL-2 polypeptide through a linker.
76. The modified IL-2 polypeptide conjugate of any of claims 36-74, wherein a water-soluble polymer, a lipid, a protein, or a peptide is directly bound to the substituted natural amino acid or unnatural amino acid of the modified IL-2 polypeptide.
77. The modified IL-2 polypeptide conjugate of any of claims 36-76, which has a half-life *in vivo* from about 5 minutes to about 10 days.
78. A pharmaceutical composition comprising an effective amount of a modified IL-2 polypeptide of any of claims 1-34, a polynucleotide of claim 35 or a modified IL-2 polypeptide conjugate of any of claims 36-77, and a pharmaceutically acceptable carrier or excipient.
79. The pharmaceutical composition of claim 78, which further comprises another active ingredient.
80. The pharmaceutical composition of claim 78 or 79, which is configured to treat or prevent a proliferation disorder in a subject.
81. The pharmaceutical composition of claim 79, wherein the another active ingredient is an anti-neoplasm substance.
82. 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 polypeptide of any of claims 1-34, a polynucleotide of claim 35, a

modified IL-2 polypeptide conjugate of any of claims 36-77, or a pharmaceutical composition of any of claims 78-81.

83. The method of claim 82, wherein the subject is a human.
84. The method of claim 82, wherein the subject is a non-human mammal.
85. The method of any of claims 82-84, which is used to treat a proliferation disorder in a subject.
86. The method of any of claims 82-84, which is used to prevent a proliferation disorder in a subject.
87. The method of any of claims 82-86, wherein the proliferation disorder is a tumor.
88. The method of any of claims 82-86, wherein the proliferation disorder is a cancer.
89. The method of claim 87 or 88, wherein the tumor or cancer is a solid tumor or cancer.
90. The method of claim 89, wherein the solid tumor or cancer is selected from the group consisting of: Chondrosarcoma, Ewing's sarcoma, Malignant fibrous histiocytoma of bone/osteosarcoma, Osteosarcoma, Rhabdomyosarcoma, Heart cancer, Astrocytoma, Brainstem glioma, Pilocytic astrocytoma, Ependymoma, Primitive neuroectodermal tumor, Cerebellar astrocytoma, Cerebral astrocytoma, Glioma, Medulloblastoma, Neuroblastoma, Oligodendroglioma, Pineal astrocytoma, Pituitary adenoma, Visual pathway and hypothalamic glioma, Breast cancer, Invasive lobular carcinoma, Tubular carcinoma, Invasive cribriform carcinoma, Medullary carcinoma, Male breast cancer, Phyllodes tumor, Inflammatory Breast Cancer, Adrenocortical carcinoma, Islet cell carcinoma (endocrine pancreas), Multiple endocrine neoplasia syndrome, Parathyroid cancer, Pheochromocytoma, Thyroid cancer, Merkel cell carcinoma, Uveal melanoma, Retinoblastoma, Anal cancer, Appendix cancer, cholangiocarcinoma, Carcinoid tumor, gastrointestinal, Colon cancer, Extrahepatic bile duct cancer, Gallbladder cancer, Gastric (stomach) cancer, Gastrointestinal carcinoid tumor, Gastrointestinal stromal tumor (GIST), Hepatocellular cancer, Pancreatic cancer islet cell, Rectal cancer, Bladder cancer, Cervical cancer, Endometrial cancer, Extragonadal germ cell tumor, Ovarian cancer, Ovarian epithelial cancer (surface epithelial-stromal tumor), Ovarian germ cell tumor, Penile cancer, Renal cell carcinoma, Renal pelvis and ureter, transitional cell cancer, Prostate cancer, Testicular cancer, Gestational trophoblastic tumor, Ureter and renal pelvis, transitional cell cancer, Urethral cancer, Uterine sarcoma, Vaginal cancer, Vulvar cancer, Wilms tumor, Esophageal cancer, Head and neck cancer, Nasopharyngeal carcinoma, Oral cancer, Oropharyngeal cancer, Paranasal sinus and nasal cavity cancer, Pharyngeal cancer, Salivary gland cancer, Hypopharyngeal cancer, Basal-cell carcinoma, Melanoma, Skin cancer (non-melanoma), Bronchial adenomas/carcinoids, Small cell lung cancer, Mesothelioma, Non-small cell lung cancer, Pleuropulmonary blastoma, Laryngeal cancer, Thymoma and thymic carcinoma, AIDS-related cancers, Kaposi sarcoma, Epithelioid hemangioendothelioma (EHE), Desmoplastic small round cell tumor and Liposarcoma.

91. The method of claim 87 or 88, wherein the tumor or cancer is a hematological malignancy.

92. The method of claim 91, wherein the hematological malignancy is selected from the group consisting of: myeloid neoplasms, Leukemias, Lymphomas, Hodgkin lymphoma, Non-Hodgkin lymphoma, Anaplastic large cell lymphoma, Angioimmunoblastic T-cell lymphoma, Hepatosplenic T-cell lymphoma, B-cell lymphoma reticuloendotheliosis, Reticulosis, Microglioma, Diffuse large B-cell lymphoma, Follicular lymphoma, Mucosa-associated lymphatic tissue lymphoma, B-cell chronic lymphocytic leukemia, Mantle cell lymphoma, Burkitt lymphoma, Mediastinal large B cell lymphoma, Waldenström's macroglobulinemia, Nodal marginal zone B cell lymphoma, Splenic marginal zone lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, Nodular lymphocyte predominant Hodgkin's lymphoma, plasma cell leukemia, Acute erythraemia and erythroleukaemia, Acute erythremic myelosis, Acute erythroid leukemia, Heilmeyer-Schöner disease, Acute megakaryoblastic leukemia, Mast cell leukemia, Panmyelosis, Acute panmyelosis with myelofibrosis, Lymphosarcoma cell leukemia, Acute leukaemia of unspecified cell type, Blastic phase chronic myelogenous leukemia, Stem cell leukemia, Chronic leukaemia of unspecified cell type, Subacute leukaemia of unspecified cell type, Accelerated phase chronic myelogenous leukemia, Acute myeloid leukemia, Polycythemia vera, Acute promyelocytic leukemia, Acute basophilic leukemia, Acute eosinophilic leukemia, Acute lymphoblastic leukemia, Acute monocytic leukemia, Acute myeloblastic leukemia with maturation, Acute myeloid dendritic cell leukemia, Adult T-cell leukemia/lymphoma, Aggressive NK-cell leukemia, B-cell prolymphocytic leukemia, B-cell chronic lymphocytic leukemia, B-cell leukemia, Chronic myelogenous leukemia, Chronic myelomonocytic leukemia, Chronic neutrophilic leukemia, Chronic lymphocytic leukemia, Hairy cell leukemia, Chronic idiopathic myelofibrosis, Multiple myeloma, Kahler's disease, Myelomatosis, Solitary myeloma, Plasma cell leukemia, Plasmacytoma, extramedullary, Malignant plasma cell tumour NOS, Plasmacytoma NOS, Monoclonal gammopathy, Multiple Myeloma, Angiocentric immunoproliferative lesion, Lymphoid granulomatosis, Angioimmunoblastic lymphadenopathy, T-gamma lymphoproliferative disease, Waldenström's macroglobulinaemia, Alpha heavy chain disease, Gamma heavy chain disease, Franklin's disease, Immunoproliferative small intestinal disease, Mediterranean disease, Malignant immunoproliferative disease, unspecified, and Immunoproliferative disease NOS.

93. The method of claim 82, wherein the disease or disorder is an immune deficiency disease or disorder.

94. The method of claim 93, wherein the immune deficiency disease or disorder is selected from the group consisting of: Agammaglobulinemia: X-Linked and Autosomal Recessive, Ataxia Telangiectasia, Chronic Granulomatous Disease and Other Phagocytic Cell Disorders, Common Variable Immune Deficiency, Complement Deficiencies, DiGeorge Syndrome, Hemophagocytic Lymphohistiocytosis (HLH), Hyper IgE Syndrome, Hyper IgM Syndromes, IgG Subclass Deficiency, Innate Immune Defects, NEMO Deficiency Syndrome, Selective IgA Deficiency, Selective IgM Deficiency, Severe Combined Immune, Deficiency and Combined Immune Deficiency, Specific Antibody Deficiency, Transient Hypogammaglobulinemia of Infancy, WHIM Syndrome (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis), Wiskott-Aldrich Syndrome, Other Antibody Deficiency

Disorders, Other Primary Cellular Immunodeficiencies, Severe combined immune deficiency (SCID), Common variable immune deficiency (CVID), Human immunodeficiency virus / acquired immune deficiency syndrome (HIV/AIDS), Drug-induced immune deficiency, Graft versus host syndrome, Primary Immune Deficiency Diseases (PIDDs), and Lymphopenia.

95. The method of any of claims 82-84, which is used to treat or prevent an autoimmune or inflammatory disease or disorder in a subject.

96. The method of claim 95, wherein the inflammatory or autoimmune diseases or disorder is selected from the group consisting of: inflammation, autoimmune disease, paraneoplastic autoimmune diseases, cartilage inflammation, fibrotic disease and/or bone degradation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), dermatomyositis, psoriatic arthritis, scleroderma, systemic lupus erythematosus, vasculitis, myolitis, polymyolitis, dermatomyolitis, osteoarthritis, polyarteritis nodosa, Wegener's granulomatosis, arteritis, ploymyalgia rheumatica, sarcoidosis, scleroderma, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjogren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus (SLE), myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, celiac disease, multiple schlerosis (MS), asthma, COPD, Guillain-Barre disease, Type I diabetes mellitus, thyroiditis (e.g., Graves' disease), Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, GVHD, and transplantation rejection.

97. The method of any of claims 82-84, which is used to treat or prevent an infectious disease or disorder in a subject.

98. The method of claim 97, wherein the infectious diseases is selected from the group consisting of: Acinetobacter infections, Actinomycosis, African sleeping sickness (African trypanosomiasis), AIDS (acquired immunodeficiency syndrome), Amoebiasis, Anaplasmosis, Angiostrongyliasis Anisakiasis, Anthrax, Arcanobacterium haemolyticum infection, Argentine hemorrhagic fever, Ascariasis, Aspergillosis Astrovirus infection, Babesiosis, Bacillus cereus infection, Bacterial meningitis, Bacterial pneumonia, Bacterial vaginosis, Bacteroides infection, Balantidiasis, Bartonellosis, Baylisascaris infection, BK virus infection, Black piedra, Blastocystosis, Blastomycosis, Bolivian hemorrhagic fever, Botulism (and Infant botulism), Brazilian hemorrhagic fever, Brucellosis, Bubonic plague, Burkholderia infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), Campylobacteriosis, Candidiasis (Moniliasis; Thrush), Capillariasis, Carrion's disease, Cat-scratch disease, Cellulitis, Chagas disease (American trypanosomiasis), Chancroid, Chickenpox, Chikungunya, Chlamydia, Chlamydophila pneumoniae infection (Taiwan acute respiratory agent or TWAR), Cholera, Chromoblastomycosis, Chytridiomycosis,

Clonorchiasis, Clostridium difficile colitis, Coccidioidomycosis, Colorado tick fever (CTF), Common cold (Acute viral rhinopharyngitis; Acute coryza), Coronavirus disease 2019 (COVID-19), Creutzfeldt–Jakob disease (CJD), Crimean-Congo hemorrhagic fever (CCHF), Cryptococcosis, Cryptosporidiosis, Cutaneous larva migrans (CLM), Cyclosporiasis, Cysticercosis, Cytomegalovirus infection, Dengue fever, Desmodermis infection, Dientamoebiasis, Diphtheria, Diphyllbothriasis, Dracunculiasis, Ebola hemorrhagic fever, Echinococcosis, Ehrlichiosis, Enterobiasis (Pinworm infection), Enterococcus infection, Enterovirus infection, Epidemic typhus, Erythema infectiosum (Fifth disease), Exanthem subitum (Sixth disease), Fascioliasis, Fasciolopsiasis, Fatal familial insomnia (FFI), Filariasis, Food poisoning by Clostridium perfringens, Free-living amebic infection, Fusobacterium infection, Gas gangrene (Clostridial myonecrosis), Geotrichosis, Gerstmann–Sträussler–Scheinker syndrome (GSS), Giardiasis, Glanders, Gnathostomiasis, Gonorrhea, Granuloma inguinale (Donovanosis), Group A streptococcal infection, Group B streptococcal infection, Haemophilus influenzae infection, Hand, foot and mouth disease (HFMD), Hantavirus Pulmonary Syndrome (HPS), Heartland virus disease, Helicobacter pylori infection, Hemolytic-uremic syndrome (HUS), Hemorrhagic fever with renal syndrome (HFRS), Hendra virus infection, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Herpes simplex, Histoplasmosis, Hookworm infection, Human bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis (HGA), Human metapneumovirus infection, Human monocytic ehrlichiosis, Human papillomavirus (HPV) infection, Human parainfluenza virus infection, Hymenolepiasis, Epstein–Barr virus infectious mononucleosis (Mono), Influenza (flu), Isosporiasis, Kawasaki disease, Keratitis, Kingella kingae infection, Kuru, Lassa fever, Legionellosis (Legionnaires' disease), Pontiac fever, Leishmaniasis, Leprosy, Leptospirosis, Listeriosis, Lyme disease (Lyme borreliosis), Lymphatic filariasis (Elephantiasis), Lymphocytic choriomeningitis, Malaria, Marburg hemorrhagic fever (MHF), Measles, Middle East respiratory syndrome (MERS), Melioidosis (Whitmore's disease), Meningitis, Meningococcal disease, Metagonimiasis, Microsporidiosis, Molluscum contagiosum (MC), Monkeypox, Mumps, Murine typhus (Endemic typhus), Mycoplasma pneumonia, Mycoplasma genitalium infection, Mycetoma, Myiasis, Neonatal conjunctivitis (Ophthalmia neonatorum), Nipah virus infection, Norovirus (children and babies), (New) Variant Creutzfeldt–Jakob disease (vCJD, nvCJD), Nocardiosis, Onchocerciasis (River blindness), Opisthorchiasis, Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Pasteurellosis, Pediculosis capitis (Head lice), Pediculosis corporis (Body lice), Pediculosis pubis (pubic lice, crab lice), Pelvic inflammatory disease (PID), Pertussis (whooping cough), Plague, Pneumococcal infection, Pneumocystis pneumonia (PCP), Pneumonia, Poliomyelitis, Prevotella infection, Primary amoebic meningoencephalitis (PAM), Progressive multifocal leukoencephalopathy, Psittacosis, Q fever, Rabies, Relapsing fever, Respiratory syncytial virus infection, Rhinosporidiosis, Rhinovirus infection, Rickettsial infection, Rickettsialpox, Rift Valley fever (RVF), Rocky Mountain spotted fever (RMSF), Rotavirus infection, Rubella, Salmonellosis, SARS (severe acute respiratory syndrome), Scabies, Scarlet fever, Schistosomiasis, Sepsis, Shigellosis (bacillary dysentery), Shingles (Herpes zoster), Smallpox (variola), Sporotrichosis, Staphylococcal food poisoning, Staphylococcal infection, Strongyloidiasis, Subacute sclerosing panencephalitis, Bejel, Syphilis, and Yaws, Taeniasis, Tetanus (lockjaw), Tinea barbae (barber's itch), Tinea capitis (ringworm of the scalp), Tinea corporis (ringworm of the body), Tinea cruris (Jock itch), Tinea manum (ringworm of the hand), Tinea nigra, Tinea pedis (athlete's foot), Tinea unguium (onychomycosis), Tinea versicolor (Pityriasis versicolor), Toxocariasis (ocular larva migrans (OLM)),

Toxocariasis (visceral larva migrans (VLM)), Toxoplasmosis, Trachoma, Trichinosis, Trichomoniasis, Trichuriasis (whipworm infection), Tuberculosis, Tularemia, Typhoid fever, Typhus fever, Ureaplasma urealyticum infection, Valley fever, Venezuelan equine encephalitis, Venezuelan hemorrhagic fever, Vibrio vulnificus infection, Vibrio parahaemolyticus enteritis, Viral pneumonia, West Nile fever, White piedra (tinea blanca), Yersinia pseudotuberculosis infection, Yersiniosis, Yellow fever, Zeaspora, Zika fever, and Zygomycosis.

99. The method of any of claims 82-99, which further comprises administering an effective amount of a second therapeutic agent for treating or preventing a proliferation disorder in a subject.

100. Use of an effective amount of a modified IL-2 polypeptide of any of claims 1-34, a polynucleotide of claim 35 or a modified IL-2 polypeptide conjugate of any of claims 36-77 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.

101. 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 of any of claims 1-34, a polynucleotide of claim 35, a modified IL-2 polypeptide conjugate of any of claims 36-77, or a pharmaceutical composition of any of claims 78-81 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.

102. 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 of any of claims 1-34, a polynucleotide of claim 35, a modified IL-2 polypeptide conjugate of any of claims 36-77, or a pharmaceutical composition of any of claims 78-81 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%.

103. The method of claim 101 or 102, wherein the modified IL-2 polypeptide of any of claims 1-34, a polynucleotide of claim 35, a modified IL-2 polypeptide conjugate of any of claims 36-77, or a pharmaceutical composition of any of claims 78-81 expands CD4⁺ T regulatory (Treg) cells by less than 20%, 15%, 10%, 5%, 1% or less in the CD3⁺ cell population compared to an expansion of CD4⁺ Treg cells in the CD3⁺ cell population contacted with 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.

104. The method of claim 101 or 102, wherein the modified IL-2 polypeptide of any of claims 1-34, the polynucleotide of claim 35, the modified IL-2 polypeptide conjugate of any of claims 36-77, or the pharmaceutical composition of any of claims 78-81 does not expand CD4⁺ Treg cells in the cell population.

105. The method of claim 101 or 102, wherein the ratio of the Teff cells to Treg cells in the cell population after incubation with the modified IL-2 polypeptide of any of claims 1-34, the polynucleotide of claim 35, the modified IL-2 polypeptide conjugate of any of claims 36-77, or the pharmaceutical composition of any of claims 78-81 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, 100:1 or more.
106. The method of any of claims 101-105, which is conducted *in vivo*.
107. The method of any of claims 101-105, which is conducted *in vitro*.
108. The method of any of claims 101-105, which is conducted *ex vivo*.
109. Use of an effective amount of a modified IL-2 polypeptide of any of claims 1-34, a polynucleotide of claim 35, or a modified IL-2 polypeptide conjugate of any of claims 36-77 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 population in a cell population.
110. The use of claim 109, which is configured for expanding a CD4⁺ helper cell, CD8⁺ effector naive and memory cell, Treg cell, Natural Killer (NK) cell, or Natural killer T (NKT) cell population in a subject.