WHAT IS CLAIMED IS:

1. An IL-2 polypeptide comprising one or more non-naturally encoded amino acids, wherein the IL-2 polypeptide has the amino acid sequence of SEQ ID NO: 2, wherein the non-naturally encoded amino acid is incorporated into position 45 of SEQ ID NO: 2, wherein a water-soluble polymer is conjugated to the IL-2 polypeptide through the non-naturally encoded amino acid via a non-cleavable linker, wherein the non-naturally encoded amino acid is para-acetyl-phenylalanine, wherein said IL-2 polypeptide has reduced interaction with its IL-2Rα receptor subunit compared to a wild-type IL-2 polypeptide.

~~2. The IL-2 of claim 1, wherein the IL-2 polypeptide is 90% homologous to SEQ ID NO: 2 or SEQ ID NO: 3.~~

~~3. The IL-2 of claim 1, wherein the IL-2 polypeptide is at least 95% homologous to SEQ ID NO: 2.~~

~~4. The IL-2 of claim 1, wherein the IL-2 polypeptide is at least 98% homologous to SEQ ID NO: 2.~~

~~5. The IL-2 of claim 1, wherein the IL-2 polypeptide is at least 99% homologous to SEQ ID NO: 2.~~

~~6. The IL-2 of claim 1, wherein the IL-2 is conjugated to one or more water-soluble polymers.~~

~~7. The IL-2 of claim 6, wherein at least one of the water-soluble polymers is linked to at least one of the non-naturally encoded amino acids.~~

~~8~~2. The IL-2 polypeptide of claim 1~~7~~, wherein the water-soluble polymer is a poly(ethylene glycol) (PEG) moiety.

3. The IL-2 polypeptide of claim 2, wherein the PEG moiety is linear or branched.

~~9~~4. The IL-2 polypeptide of claim ~~8~~2 or claim 3, wherein the PEG moiety has a molecular weight between 0.1 kDa and 100 kDa, preferably between 0.1 and 50 kDa, more preferably between 10 and 50.

5. The IL-2 polypeptide of any one of claims 1-4, wherein the non-cleavable linker is an oxime linkage.

~~10. The IL-2 of claim 1, wherein the non-naturally encoded amino acid is substituted at a position selected from the group consisting of residues before position 1 (i.e. at the N-terminus), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or added to the carboxyl terminus of the protein, and any combination thereof.~~

~~11. The IL-2 of claim 10, wherein the IL-2 comprises one or more amino acid substitution, addition or deletion that modulates affinity of the IL-2 polypeptide for its IL2Rα receptor subunit compared to wild-type IL-2.~~

~~12. The IL-2 of claim 10, wherein the IL-2 comprises one or more amino acid substitution, addition or deletion that increases the stability or solubility of the IL-2.~~

~~13. The IL-2 of claim 10, wherein the IL-2 comprises one or more amino acid substitution, addition or deletion that increases the expression of the IL-2 polypeptide in a recombinant host cell or synthesized in vitro.~~

~~14. The IL-2 of claim 10, wherein non-naturally encoded amino acid is substituted at a position selected from the group consisting of residues 3, 35, 37, 38, 41, 42, 43, 44, 45, 61, 62, 64, 65, 68, 72, and 107, and any combination thereof.~~

~~15. The IL-2 of claim 10, wherein the non-naturally encoded amino acid is reactive toward a linker, polymer, or biologically active molecule that is otherwise unreactive toward any of the 20 common amino acids in the polypeptide.~~

16. The IL-2 of claim 10, wherein the non-naturally encoded amino acid comprises a carbonyl group, an aminooxy group, a hydrazine group, a hydrazide group, a semicarbazide group, an azide group, or an alkyne group.

17. The IL-2 of claim 16, wherein the non-naturally encoded amino acid comprises a carbonyl group.

186. The IL-2 polypeptide of any one of claims 1-510, wherein the IL-2 polypeptide is further linked to a biologically active molecule, a cytotoxic agent, a water-soluble polymer, or an immunostimulatory agent, optionally wherein the biologically active molecule, cytotoxic agent, or immunostimulatory agent is linked to the IL-2 polypeptide by a linker, further optionally wherein the linker is a cleavable or non-cleavable linker.

7. The IL-2 polypeptide of any one of claims 1-6, wherein the IL-2 polypeptide is produced in CHO cells.

8. The IL-2 polypeptide of claim 1, wherein the water-soluble polymer is a PEG moiety, wherein the PEG moiety is linear, wherein the PEG moiety has a molecular weight of between 10 kDa and 50 kDa, and wherein the non-cleavable linker is an oxime linkage.

9. The IL-2 polypeptide of any one of claims 1-8, wherein the IL-2 polypeptide provides increased *in vivo* half life, wherein the *in vivo* half-life of the IL-2 polypeptide is increased at least about 5-fold relative to the unmodified polypeptide.

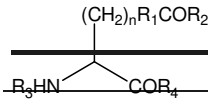
19. The IL-2 of claim 18, wherein the conjugated IL-2 is attached to one or more water-soluble polymers.

20. The IL-2 of claim 18, wherein the biologically active molecule, cytotoxic agent, or immunostimulatory agent is linked to the IL-2 by a linker.

21. The IL-2 of claim 18, wherein the biologically active molecule, cytotoxic agent, or immunostimulatory agent is linked to the IL-2 by a cleavable or non-cleavable linker.

22. The IL-2 of claim 18, wherein the biologically active molecule, cytotoxic agent, or immunostimulatory agent is conjugated directly to one or more of the non-naturally encoded amino acids in the IL-2.

23. The IL-2 of claim 10, wherein the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R1 is an alkyl, aryl, substituted alkyl, or substituted aryl; R2 is H, an alkyl, aryl, substituted alkyl, and substituted aryl; and R3 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R4 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

24. The IL-2 of claim 23, wherein the non-naturally encoded amino acid comprises an aminooxy group.

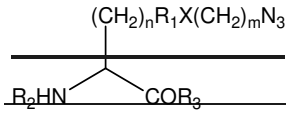
25. The IL-2 of claim 23, wherein the non-naturally encoded amino acid comprises a hydrazide group.

26. The IL-2 of claim 23, wherein the non-naturally encoded amino acid comprises a hydrazine group.

27. The IL-2 of claim 23, wherein the non-naturally encoded amino acid residue comprises a semicarbazide group.

28. The IL-2 polypeptide of claim 23, wherein the non-naturally encoded amino acid residue comprises an azide group.

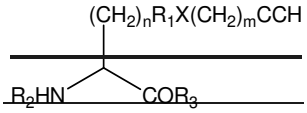
29. The IL-2 of claim 1, wherein the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R1 is an alkyl, aryl, substituted alkyl, substituted aryl or not present; X is O, N, S or not present; m is 0-10; R2 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R3 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

30. The IL-2 of claim 29, wherein the non-naturally encoded amino acid comprises an alkyne group.

31. The IL-2 of claim 1, wherein the non-naturally encoded amino acid has the structure:



wherein n is 0-10; R1 is an alkyl, aryl, substituted alkyl, or substituted aryl; X is O, N, S or not present; m is 0-10, R2 is H, an amino acid, a polypeptide, or an amino terminus modification group, and R3 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

32. The IL-2 of claim 7, wherein the water-soluble polymer has a molecular weight of between about 0.1 kDa and about 100 kDa.

33. The IL-2 polypeptide of claim 32, wherein the water-soluble polymer has a molecular weight of between about 0.1 kDa and about 50 kDa.

34. The IL-2 of claim 16, wherein the aminooxy, hydrazine, hydrazide or semicarbazide group is linked to the water-soluble polymer through an amide linkage.

35. The IL-2 of claim 19, which is made by reacting a water-soluble polymer comprising a carbonyl group with a polypeptide comprising a non-naturally encoded amino acid that comprises an aminooxy, a hydrazine, a hydrazide or a semicarbazide group.

36. The IL-2 of claim 1, wherein the IL-2 is glycosylated.

37. The IL-2 of claim 1, wherein the IL-2 polypeptide further comprises a linker, polymer, or biologically active molecule linked to the polypeptide via the non-naturally encoded amino acid.

38. The IL-2 of claim 37, wherein the IL-2 polypeptide wherein the linker, polymer, or biologically active molecule linked to the polypeptide via a saccharide moiety.

~~39~~10. A method of making the IL-2 polypeptide of any one claims 1 to 9, the method comprising contacting an isolated IL-2 polypeptide ~~comprising a non-naturally encoded amino acid~~of as defined in any one of claims 1 to 9 with a ~~linker, ~~water-soluble polymer, ~~or biologically active molecule~~wherein the water-soluble polymer comprises~~ing~~ a moiety that reacts with the non-naturally encoded amino acid, and wherein the IL-2 polypeptide is obtained by expression in mammalian cells or eukaryotic cells, such as CHO cells.

11. A method of making the IL-2 polypeptide of any one of claims 1-9, the method comprising contacting the IL-2 polypeptide as defined in any of claims 1-9 with a water-soluble polymer, wherein the water-soluble polymer comprises a moiety that reacts with the non-naturally encoded amino acid, and wherein the IL-2 polypeptide is obtained by expression in mammalian cells or eukaryotic cells, such as CHO cells.

~~40~~12. The method of claim ~~39~~10 or claim 11, wherein the water-soluble polymer ~~comprises a moiety selected from a group consisting of a water soluble polymer and~~is a poly(ethylene glycol) (PEG) moiety.

13. The method of claim 12, wherein the PEG moiety is linear or branched.

14. The method of claim 12 or claim 13, wherein the PEG moiety has a molecular weight between 0.1 kDa and 100 kDa, preferably between 0.1 and 50 kDa, more preferably between 10 and 50.

~~41. The method of claim 39, wherein the non-naturally encoded amino acid comprises a carbonyl group, an aminooxy group, a hydrazide group, a hydrazine group, a semicarbazide group, an azide group, or an alkyne group.~~

~~42. The method of claim 39, wherein the non-naturally encoded amino acid comprises a carbonyl moiety and the linker, polymer, or biologically active molecule comprises an aminooxy, a hydrazine, a hydrazide or a semicarbazide moiety.~~

~~43. The method of claim 39, wherein the aminooxy, hydrazine, hydrazide or semicarbazide moiety is linked to the linker, polymer, or biologically active molecule through an amide linkage.~~

~~44. The method of claim 39, wherein the non-naturally encoded amino acid comprises an alkyne moiety and the linker, polymer, or biologically active molecule comprises an azide moiety.~~

~~45. The method of claim 39, wherein the non-naturally encoded amino acid comprises an azide moiety and the linker, polymer, or biologically active molecule comprises an alkyne moiety.~~

~~46. The IL-2 polypeptide of claim 7, wherein the water soluble polymer is a poly(ethylene glycol) moiety.~~

~~47. The IL-2 polypeptide of claim 46, wherein the poly(ethylene glycol) moiety is a branched or multiarmed polymer.~~

15. An IL-2 polypeptide obtained or obtainable by the method of any one of claims 10-14.

~~48~~16. A composition comprising the IL-2 polypeptide of any one of claims 1-9~~10~~ and a pharmaceutically acceptable carrier, wherein the IL-2 polypeptides in the composition are homogenous.

17. The IL-2 polypeptide of any one of claims 1-9, or the composition of claim 16, for use in treating a patient having a condition, disease or disorder modulated by IL-2.

18. The IL-2 polypeptide or composition for use according to claim 17, wherein the condition or disease modulated by IL-2 is cancer.

19. Use of the IL-2 polypeptide of any one of claims 1-9, or the composition of claim 17, in the manufacture of a medicament for treating a patient having a condition, disease or disorder modulated by IL-2.

20. The use according to claim 19, wherein the condition or disease modulated by IL-2 is cancer.

~~49. The composition of claim 48, wherein the non-naturally encoded amino acid is linked to a water-soluble polymer.~~

50. A method of treating a patient having a disorder modulated by IL-2 comprising administering to the patient a therapeutically-effective amount of the composition of claim 42.

51. A composition comprising the IL-2 of claim 10 conjugated to a biologically active molecule with a pharmaceutically acceptable carrier.

52. A composition comprising the IL-2 of claim 10 further comprising a linker and a conjugate with a pharmaceutically acceptable carrier.

53. A method of making an IL-2 comprising a non-naturally encoded amino acid, the method comprising, culturing cells comprising a polynucleotide or polynucleotides encoding an IL-2 polypeptide comprising a selector codon, an orthogonal RNA synthetase and an orthogonal tRNA under conditions to permit expression of the IL-2 polypeptide comprising a non-naturally encoded amino acid; and purifying said polypeptide.

54. A method of modulating serum half-life or circulation time of an IL-2 polypeptide, the method comprising substituting one or more non-naturally encoded amino acids for any one or more naturally occurring amino acids in said polypeptide.

55. An IL-2 polypeptide comprising one or more amino acid substitution, addition or deletion that increases the expression of the IL-2 polypeptide in a recombinant host cell.

56. An IL-2 polypeptide comprising at least one linker, polymer, or biologically active molecule, wherein said linker, polymer, or biologically active molecule is attached to the polypeptide through a functional group of a non-naturally encoded amino acid ribosomally incorporated into the polypeptide.

57. An IL-2 polypeptide comprising a linker, polymer or biologically active molecule that is attached to one or more non-naturally encoded amino acids wherein said non-naturally encoded amino acid is ribosomally incorporated into the polypeptide at pre-selected sites.

58. A method for reducing the number of tumor cells in a human diagnosed with cancer, comprising administering to a human in need of such reduction a pharmaceutical composition comprising a PEG-IL-2 of claim 56.

59. The method of claim 58, wherein the conjugate is administered at a dose of about 0.1 μ /kg to about 50 μ/kg.

60. The IL-2 of any of claims 1-38, 46-47 and 55-57, wherein the IL-2 further comprises at least one natural amino acid substitution at one or more positions selected from the group consisting of residues 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133.

61. The method of any of claims 39-45, 53-54, and 58-59 or the composition of any of claims 48-49 wherein the method or composition further comprises at least one natural amino acid substitution at one or more positions selected from the group consisting of residues 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133.

62. The IL-2 of claim 60, the method or the composition of claim 61, wherein the natural amino acid substitution is at positions 38, 46 and/or 65.

63. The IL-2 of claim 60, the method or the composition of claim 61, wherein the natural amino acid substitution is at positions 38 and 46.

64. The IL-2 of claim 60, the method or the composition of claim 61, wherein the natural amino acid substitution is at positions 38 and 65.

65. The IL-2 or the method or the composition of any of claims 62-64, wherein the natural amino acid substitution at position 38 is an alanine.

~~66. The IL-2 or the method or the composition of any of claim 62-63, wherein the natural amino acid substitution at position 46 is a leucine or isoleucine.~~

~~67. The IL-2 or the method or the composition of any of claim 62 or 64, wherein the natural amino acid substitution at position 65 is an arginine.~~