

WHAT IS CLAIMED:

1. An interleukin 2 (IL-2) conjugate comprising an IL-2 polypeptide comprising an amino acid sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprising:
- 5 (i) one or more amino acid substitutions that reduce(s) affinity of the IL-2 polypeptide for the human IL-2 receptor $\alpha\beta\gamma_C$ trimer (IL-2R $\alpha\beta\gamma_C$) relative to wild-type human IL-2; and
- (ii) a substitution of an amino acid at or near the N-terminus of the IL-2 polypeptide with a non-natural amino acid conjugated to a nonpeptidic, water-soluble polymer or
- 10 an insertion of a non-natural amino acid conjugated to a nonpeptidic, water-soluble polymer at or near the N-terminus of the IL-2 polypeptide;
- wherein the IL-2 polypeptide has substantially similar binding affinity for the human IL-2 receptor $\beta\gamma_C$ dimer (IL-2R $\beta\gamma_C$) relative to wild-type human IL-2.
- 15 2. The IL-2 conjugate of claim 1, wherein the IL-2 polypeptide comprising at least 80%, 85%, 90%, 95%, 98%, or 99% identity with the amino acid sequence set forth in SEQ ID NO: 53 with the proviso that the IL-2 polypeptide comprises at least amino acids E15, H16, L19, D20, D84, N88, V91, Q126, T123, and I129, wherein the amino acid positions correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53.
- 20 3. The IL-2 conjugate of claim 1, wherein the IL-2 polypeptide conjugate has no detectable binding to the human IL-2 receptor α monomer (IL-2R α) as determined by a Surface Plasmon Resonance assay.
- 25 4. The IL-2 conjugate of claim 1, wherein the one or more amino acid substitutions are independently selected from the group consisting of K34, T36, R37, T40, F41, K42, F43, Y44, E60, E61, K63, P64, E67, L71, M103, C104, and Y106, wherein the amino acid substitution positions correspond to the position of the amino acid in the amino acid sequence set forth in SEQ ID NO: 53.
- 30 5. The IL-2 conjugate of claim 3, wherein the one or more amino acid substitutions in the IL-2 polypeptide are at positions R37 and F41.

6. The IL-2 conjugate of claim 4, wherein the amino acid substitutions in the IL-2 polypeptide are R37A and F41K.

7. The IL-2 conjugate of claim 1, wherein the IL-2 polypeptide further includes a substitution of the cysteine residue at position 124 with an amino acid selected from the group consisting of A and S, wherein the amino acid position corresponds to the position of the amino acid in the amino acid sequence set forth in SEQ ID NO: 53.

8. The IL-2 conjugate of claim 1, wherein the IL-2 polypeptide further includes an N-terminal alanine residue.

9. The IL-2 conjugate of claim 1, wherein the non-natural amino acid is substituted for an amino acid at position P1, T2, S3, S4, S5, T6, K7, K8, or T9 or linked to the N-terminal amino acid by an amide linkage.

10. The IL-2 conjugate of claim 9, wherein the non-natural amino acid is located at the amino acid position corresponding to position 4 of the amino acid sequence set forth in SEQ ID NO: 2.

11. The IL-2 conjugate of claim 1, wherein the non-natural amino acid comprises a functional group and the water-soluble polymer is linked to a reactive group that is capable of reacting with the functional group to form a covalent linkage.

12. The IL-2 conjugate of claim 1, wherein the non-natural amino acid is selected from the group consisting of p-azidomethyl-L-phenylalanine, p-azido-L-phenylalanine, p-acetyl-L-phenylalanine, N6-azidoethoxy-L-lysine, N6-propargylethoxy-L-lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxycarbonyllysine, 2-amino-8-oxononanoic acid, 2-amino-8-oxooctanoic acid, O-methyl-L-tyrosine, L-3-(2-naphthyl)alanine, 3-methyl-phenylalanine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, tri-O-acetyl-GlcNAc-serine, L-Dopa, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-benzoyl-L-phenylalanine, L-phosphoserine, phosphoserine, phosphotyrosine, p-iodo-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, p-propargyloxy-phenylalanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)sulfonyl)propanoic acid, 2-amino-3-(phenylsulfonyl)propanoic,

selenocysteine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, and p-propargyloxyphenylalanine.

13. The IL-2 conjugate of claim 1, wherein the non-natural amino acid is p-azidomethyl-L-phenylalanine.

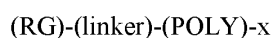
14. The IL-2 conjugate of claim 1, wherein the nonpeptidic, water-soluble polymer has an average molecular weight between 1 kDa and 100 kDa.

15. The IL-2 conjugate of claim 1, wherein the nonpeptidic, water-soluble polymer has an average molecular weight of about 30 kDa.

16. The IL-2 conjugate of claim 1, wherein the nonpeptidic, water-soluble polymer is 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.

17. The IL-2 conjugate of claim 1, wherein the nonpeptidic, water-soluble polymer is a linear or branched PEG.

18. The IL-2 conjugate of claim 11, wherein the nonpeptidic, water-soluble polymer linked to the reactive group has the formula:

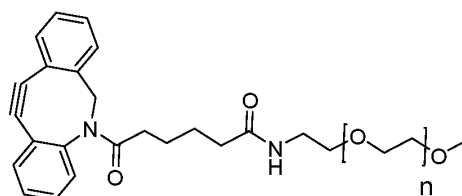


wherein RG is a reactive group that is capable of forming a covalent linkage with the functional group of a non-natural amino acid; linker is a covalent bond or a substituted or non-substituted C₁₋₂₀ alkyl; POLY is a nonpeptidic, water-soluble polymer; and x is an alcohol or methyl group at the terminus of the POLY.

19. The IL-2 conjugate of claim 11, wherein the reactive group of the nonpeptidic, water-soluble polymer comprises an alkyne and the functional group of the non-

natural amino acid comprises an azide or the reactive group of the nonpeptidic, water-soluble polymer comprises an azide and the functional group of the non-natural amino acid comprises an alkyne.

- 5 20. The IL-2 conjugate of claim 18, wherein the nonpeptidic, water-soluble polymer linked to the reactive group has the formula:

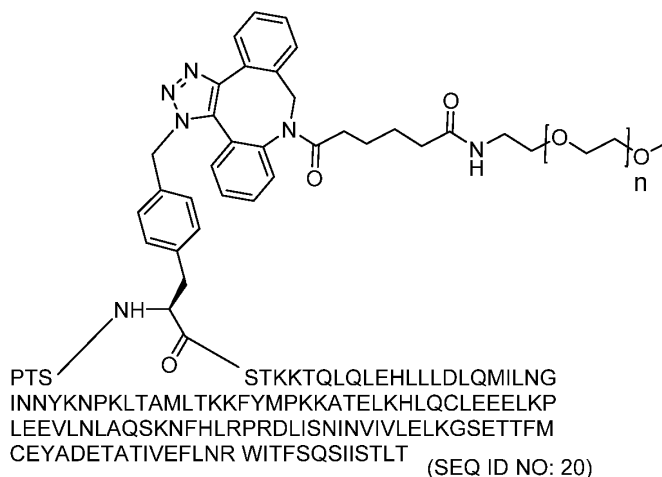


wherein n is about 681.

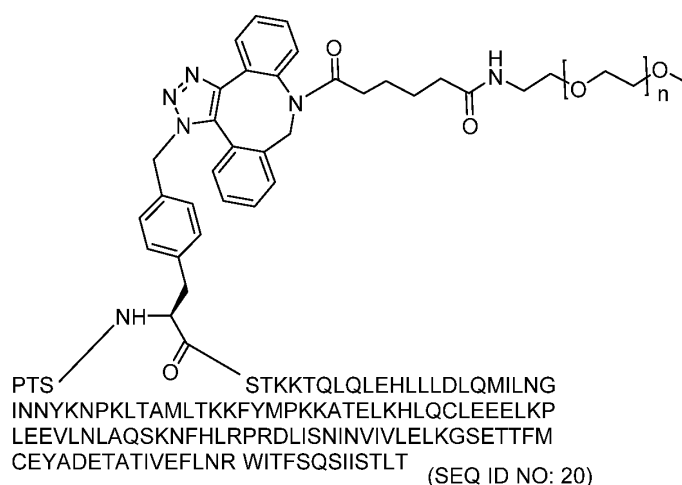
- 10 21. The IL-2 conjugate of claim 1, wherein the IL-2 conjugate comprises the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

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22. An interleukin 2 (IL-2) conjugate comprising the formula



wherein n is about 681, or a regioisomer thereof comprising the formula



wherein n is about 681.

- 5 23. A composition comprising:
 the IL-2 conjugate of any one of the foregoing claims and a pharmaceutically
 acceptable carrier or excipient.
- 10 24. A method for treating a proliferative disease or cancer in an individual,
 comprising:
 administering a therapeutically effective amount of the IL-2 conjugate of any one
 of claims 1-22 or composition of claim 23 to an individual in need thereof to treat the
 proliferative disease or cancer in the individual.
- 15 25. A combination therapy for treating a proliferative disease or cancer in an
 individual, comprising:
 administering a therapeutically effective amount of the IL-2 conjugate of any one
 of claims 1-22 or composition of claim 23 to an individual in need thereof, and
 administering a therapeutically effective amount of a therapeutic agent to the
 20 individual, to treat the proliferative disease or cancer in the individual.
26. The combination therapy of claim 25, wherein the therapeutic agent is an
 anti-PD1 antibody or anti-PDL1 antibody.

27. The combination therapy of claim 25, wherein the IL-2 conjugate or composition is administered before the therapeutic agent is administered.

28. The combination therapy of claim 25, wherein the IL-2 conjugate or composition is administered after the therapeutic agent is administered.

29. The combination therapy of claim 25, wherein the IL-2 conjugate or composition is administered concurrently with the therapeutic agent.

30. Use of the IL-2 conjugate of any one of claims 1-22 or composition of claim 23 for the treatment of a proliferative disease or cancer.

31. Use of the IL-2 conjugate of any one of claims 1-22 or composition of claim 23 and a therapeutic agent for the treatment of a proliferative disease or cancer.

32. The use of claim 31, wherein the therapeutic agent is an anti-PD1 antibody or anti-PDL1 antibody.

33. Use of the IL-2 conjugate of any one of claims 1-22 or composition of claim 23 for the manufacture of a medicament for the treatment of a proliferative disease or cancer.

34. An interleukin 2 (IL-2) conjugate comprising an IL-2 polypeptide comprising an amino acid sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprising:

(i) one or more amino acid substitutions selected from the group consisting of K34, T36, R37, T40, F41, K42, F43, Y44, E60, E61, K63, P64, E67, L71, M103, C104, and Y106, ; and

(ii) a substitution of an amino acid at or near the N-terminus of the IL-2 polypeptide with a non-natural amino acid conjugated to a nonpeptidic, water-soluble polymer or an insertion of a non-natural amino acid conjugated to a nonpeptidic, water-soluble polymer at or near the N-terminus of the IL-2 polypeptide, wherein the amino acid positions correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53.

35. The IL-2 conjugate of claim 34, the IL-2 polypeptide amino acid sequence comprises at least 80%, 85%, 90%, 95%, 98%, or 99% identity with amino acid sequence set forth in SEQ ID: 2 with the proviso that the IL-2 polypeptide comprises at least amino acids E15, H16, L19, D20, D84, N88, V91, Q126, T123, and I129, wherein the amino acid positions
5 correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53.

36. The IL-2 conjugate of claim 34, wherein the one or more amino acid substitutions in the IL-2 polypeptide are at positions R37 and F41.

10 37. The IL-2 conjugate of claim 36, wherein the amino acid substitutions in the IL-2 polypeptide are R37A and F41K.

38. The IL-2 conjugate of claim 34, wherein the IL-2 polypeptide further includes a substitution of the cysteine residue at position 124 with an amino acid selected from
15 the group consisting of A and S.

39. The IL-2 conjugate of claim 34, wherein the IL-2 polypeptide further includes an N-terminal alanine residue.

20 40. The IL-2 conjugate of claim 34, wherein the non-natural amino acid is substituted for an amino acid at position P1, T2, S3, S4, S5, T6, K7, K8, or T9 or linked to the N-terminal amino acid by an amide linkage.

41. The IL-2 conjugate of claim 40, wherein the non-natural amino acid is
25 located at the amino acid position corresponding to position 4 of the amino acid sequence set forth in SEQ ID NO: 2.

42. The IL-2 conjugate of claim 34, wherein the non-natural amino acid comprises a functional group and the water-soluble polymer is linked to a reactive group that is
30 capable of reacting with the functional group to form a covalent linkage.

43. The IL-2 polypeptide conjugate of claim 34, wherein the non-natural amino acid is selected from the group consisting of p-azidomethyl-L-phenylalanine, p-azido-L-phenylalanine, p-acetyl-L-phenylalanine, N6-azidoethoxy-L-lysine, N6-propargylethoxy-L-

lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxy carbonyllysine, 2-amino-8-oxononanoic acid, 2-amino-8-oxooctanoic acid, O-methyl-L-tyrosine, L-3-(2-naphthyl)alanine, 3-methyl-phenylalanine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, tri-O-acetyl-GlcNAc-serine, L-Dopa, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-benzoyl-L-phenylalanine, L-phosphoserine, phosphoserine, phosphotyrosine, p-iodo-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, p-propargyloxy-phenylalanine, 2-amino-3-((2-((3- (benzyloxy)-3-oxopropyl)amino)ethyl)selenyl)propanoic acid, 2-amino-3- (phenylselenyl)propanoic, selenocysteine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, and p-propargyloxyphenylalanine.

44. The IL-2 conjugate of claim 34, wherein the non-natural amino acid is p-azidomethyl-L-phenylalanine.

45. The IL-2 conjugate of claim 34, wherein the nonpeptidic, water-soluble polymer has an average molecular weight between 1 kDa and 100 kDa.

46. The IL-2 conjugate of claim 34, wherein the nonpeptidic, water-soluble polymer has an average molecular weight of about 30 kDa.

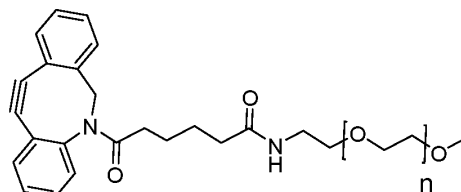
47. The IL-2 conjugate of claim 34, wherein the nonpeptidic, water-soluble polymer is polyethylene glycol (PEG), poly(propylene glycol) (PPG), copolymers of ethylene glycol and propylene glycol, poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof.

48. The IL-2 conjugate of claim 34, wherein the nonpeptidic, water-soluble polymer is a linear or branched PEG.

49. The IL-2 conjugate of claim 42, wherein the reactive group of the nonpeptidic, water-soluble polymer comprises an alkyne and the functional group of the non-natural amino acid comprises an azide or the reactive group of the nonpeptidic, water-soluble

polymer comprises an azide and the functional group of the non-natural amino acid comprises an alkyne.

50. The IL-2 conjugate of claim 48, wherein the nonpeptidic, water-soluble polymer, which comprises the alkyne, has the formula:



wherein n is about 681.

51. The IL-2 conjugate of claim 34, wherein the IL-2 conjugate comprises the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

52. A composition comprising:
the IL-2 conjugate of any one of claims 34-51 and a pharmaceutically acceptable carrier or excipient.

53. A method for treating a proliferative disease or cancer in an individual, comprising:
administering a therapeutically effective amount of the IL-2 conjugate of claims 34-51 or the composition of claim 52 to an individual in need thereof to treat the proliferative disease or cancer in the individual.

54. A combination therapy for treating a proliferative disease or cancer in an individual, comprising:
administering a therapeutically effective amount of the IL-2 conjugate of claims 34-51 or the composition of claim 52 to an individual in need thereof, and
administering a therapeutically effective amount of a therapeutic agent to the individual, to treat the proliferative disease or cancer in the individual.

55. The combination therapy of claim 54, wherein the therapeutic agent is an anti-PD1 antibody or anti-PDL1 antibody.

5 56. The combination therapy of claim 54, wherein the IL-2 conjugate or composition is administered before the therapeutic agent is administered.

57. The combination therapy of claim 54, wherein the IL-2 conjugate or composition is administered after the therapeutic agent is administered.

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58. The combination therapy of claim 54, wherein the IL-2 conjugate or composition is administered concurrently with the therapeutic agent.

59. Use of the IL-2 conjugate of claims 34-51 or the composition of claim 52 for the treatment of a proliferative disease or cancer.

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60. Use of the IL-2 conjugate of claims 34-51 or the composition of claim 52 and a therapeutic agent for the treatment of a proliferative disease or cancer.

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61. The use of claim 60, wherein the therapeutic agent is an anti-PD1 antibody or anti-PDL1 antibody.

62. Use of the IL-2 conjugate of claims 34-51 or the composition of claim 52 for the manufacture of a medicament for the treatment of a proliferative disease or cancer.

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63. An IL-2 conjugate comprising the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

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64. A pharmaceutical composition comprising the IL-2 conjugate of claim 63 and a pharmaceutically acceptable carrier or excipient.

65. An IL-2 moiety comprising an amino acid sequence set forth in SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, or SEQ ID NO: 52.

5 66. An interleukin 2 (IL-2) conjugate comprising an IL-2 polypeptide comprising an amino acid sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprising:
a substitution of an amino acid at or near the N-terminus of the IL-2 polypeptide with a non-natural amino acid conjugated to a nonpeptidic, water-soluble polymer or an insertion
10 of a non-natural amino acid conjugated to a nonpeptidic, water-soluble polymer at or near the N-terminus of the IL-2 polypeptide, with the proviso that the IL-2 polypeptide comprises at least amino acids E15, H16, L19, D20, K34, T36, R37, T40, F41, K42, F43, Y44, E60, E61, K63, P64, E67, L71, D84, N88, V91, M103, C104, Y106, Q126, T123, and I129, wherein the amino acid positions correspond to the positions set forth in the amino acid sequence of SEQ ID NO:
15 53.

67. The IL-2 conjugate of claim 66, wherein the IL-2 polypeptide further includes a substitution of the cysteine residue at position 124 with an amino acid selected from the group consisting of A and S.
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68. The IL-2 conjugate of claim 66, wherein the IL-2 polypeptide further includes an N-terminal alanine residue.

69. The IL-2 conjugate of claim 66, wherein the non-natural amino acid is
25 substituted for an amino acid at position P1, T2, S3, S4, S5, T6, K7, K8, or T9 or linked to the N-terminal amino acid by an amide linkage.

70. The IL-2 conjugate of claim 69, wherein the non-natural amino acid is located at the amino acid position corresponding to position 4 of the amino acid sequence set
30 forth in SEQ ID NO: 2.

71. The IL-2 conjugate of claim 66, wherein the non-natural amino acid comprises a functional group and the water-soluble polymer is linked to a reactive group that is capable of reacting with the functional group to form a covalent linkage.

72. The IL-2 polypeptide conjugate of claim 66, wherein the non-natural amino acid is selected from the group consisting of p-azidomethyl-L-phenylalanine, p-azido-L-phenylalanine, p-acetyl-L-phenylalanine, N6-azidoethoxy-L-lysine, N6-propargylethoxy-L-lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxycarbonyllysine, 2-amino-8-oxononanoic acid, 2-amino-8-oxooctanoic acid, O-methyl-L-tyrosine, L-3-(2-naphthyl)alanine, 3-methyl-phenylalanine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, tri-O-acetyl-GlcNAc-serine, L-Dopa, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-benzoyl-L-phenylalanine, L-phosphoserine, phosphoserine, phosphotyrosine, p-iodo-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, p-propargyloxy-phenylalanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)sulfonyl)propanoic acid, 2-amino-3-(phenylsulfonyl)propanoic, selenocysteine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, and p-propargyloxyphenylalanine.

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73. The IL-2 conjugate of claim 66, wherein the non-natural amino acid is p-azidomethyl-L-phenylalanine.

74. The IL-2 conjugate of claim 66, wherein the nonpeptidic, water-soluble polymer has an average molecular weight between 1 kDa and 100 kDa.

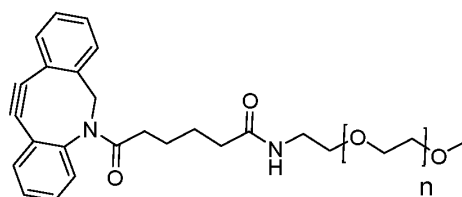
75. The IL-2 conjugate of claim 66, wherein the nonpeptidic, water-soluble polymer has an average molecular weight of about 30 kDa.

76. The IL-2 conjugate of claim 66, wherein the nonpeptidic, water-soluble polymer is 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.

77. The IL-2 conjugate of claim 66, wherein the nonpeptidic, water-soluble polymer is a linear or branched PEG.

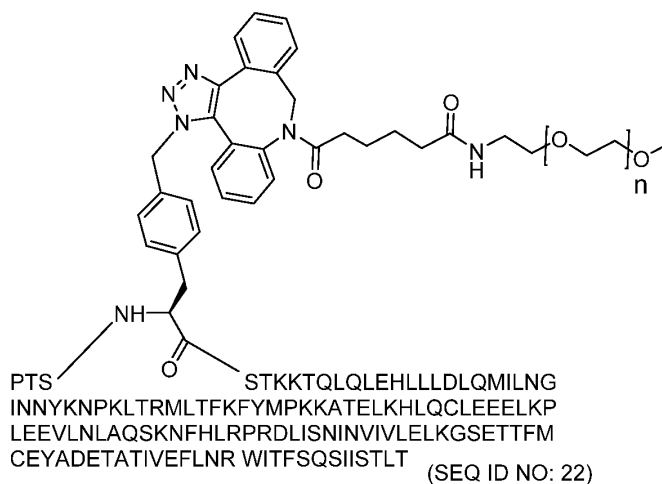
78. The IL-2 conjugate of claim 71, wherein the reactive group of the nonpeptidic, water-soluble polymer comprises an alkyne and the functional group of the non-natural amino acid comprises an azide or the reactive group of the nonpeptidic, water-soluble polymer comprises an azide and the functional group of the non-natural amino acid comprises an alkyne.

79. The IL-2 conjugate of claim 78, wherein the nonpeptidic, water-soluble polymer, which comprises the alkyne, has the formula:

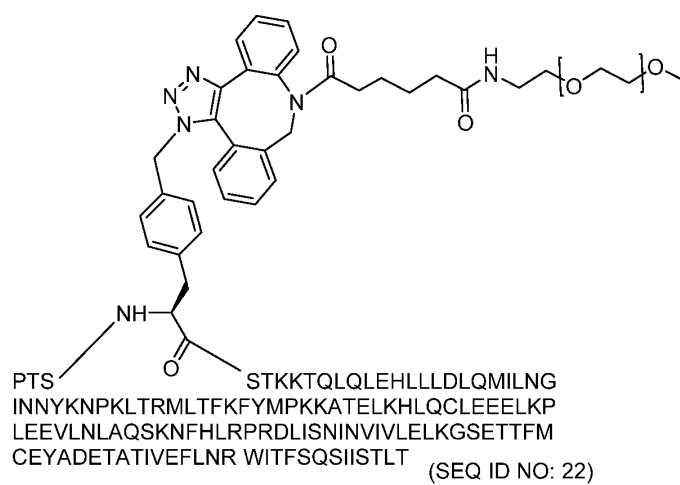


wherein n is about 681.

80. The IL-2 conjugate of claim 66, wherein the IL-2 conjugate comprises the formula:



wherein n is about 681, or its regioisomer having the formula



wherein n is about 681.