BRIEF SUMMARY

The present disclosure provides herein interleukin 2 (IL-2) polypeptides having at least one or more amino acid substitutions that bias binding to the IL-2 receptor $\beta\gamma_C$ dimer (IL-2R$\beta\gamma_C$) over the IL-2 receptor $\alpha\beta\gamma_C$ trimer (IL-2R$\alpha\beta\gamma_C$), a non-natural amino acid at or near the N-terminus of the IL-2 polypeptide conjugated to a nonpeptidic, water-soluble polymer, and an amino acid sequence with at least 80% identity otherwise to the amino acid sequence wild-type or native human IL-2. These IL-2 conjugates have a binding affinity to the IL-2R$\beta\gamma_C$ that is substantially similar to that of native IL-2 and has reduced or no detectable binding affinity for the IL-2Rα compared to native IL-2 as determined by Surface Plasmon Resonance (SPR) assay. Thus, these IL-2 conjugates have reduced toxicity compared to compared to commercially available IL-2 polypeptides and a plasma half-life of at least five times that of commercially available IL-2 polypeptides.

The present disclosure provides 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 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 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.

In one aspect, the present disclosure provides an interleukin 2 (IL-2) conjugate comprising an IL-2 polypeptide comprising an amino acid sequence with at least 80% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprising: (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 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.

In further examples, 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 correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53.

In further examples, the IL-2 polypeptide amino acid sequence comprises at least 80% 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 correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53. In further examples, the IL-2 polypeptide amino acid sequence

comprises at least 90% 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 correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53. In further examples, the IL-2 polypeptide amino acid sequence comprises at least 95% 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 correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53. In further examples, the IL-2 polypeptide amino acid sequence comprises at least 98% 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 correspond to the positions set forth in the amino acid sequence of SEQ ID NO: 53.

In a further example, the IL-2 polypeptide conjugate has reduced or no detectable binding to the human IL-2 receptor α monomer (IL-2Rα) compared to native IL-2 as determined by an SPR assay.

In a further example, the one or more amino acid substitutions are independently selected from the group consisting of K34, T36, R37, T40, F41, K42, F43, Y44, E60, , 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. In a further example, the one or more amino acid substitutions in the IL-2 polypeptide are at positions R37 and F41. In a further example, the amino acid substitutions in the IL-2 polypeptide are R37A and F41K. In a further example, 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. In a further example, the IL-2 polypeptide further includes an N-terminal alanine residue or methionine residue. In a further example, the IL-2 polypeptide further includes a C-terminal HIS6 tag.

In a further example, the non-natural amino acid is substituted for an amino acid corresponding to a position within the first 10 amino acids of the amino acid sequence as set forth in SEQ ID NO: 2 or inserted within said sequence. In a further example, the non-natural amino acid is substituted for an amino acid corresponding to a position within the sequence from P1 to Q10 of the amino acid sequence as set forth in SEQ ID NO: 2 or inserted within said sequence between P1 through Q10. In a further example, 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. In a further example, the non-natural amino acid is inserted after the amino acid at position P1, T2, S3, S4, S5, T6, K7, K8, or T9. In a further example, 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.

In a further example, the non-natural amino acid comprises a functional group and the nonpeptidic, water-soluble polymer is linked to a reactive group that is capable of reacting with the functional group to form a covalent linkage. In a further example, 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, phosphonoserine, phosphonotyrosine, 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)selanyl)propanoic acid, 2-amino-3- (phenylselanyl)propanoic, selenocysteine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, and p-propargyloxyphenylalanine. In a further example, the non-natural amino acid is p-azidomethyl-L-phenylalanine.

In a further example, the nonpeptidic, water-soluble polymer has an average molecular weight between 1 kDa and 100 kDa. In a further example, the nonpeptidic, water-soluble polymer has an average molecular weight of about 30 kDa. In a further example, 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(a-hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof. In a further example, the nonpeptidic, water-soluble polymer is a linear or branched PEG.

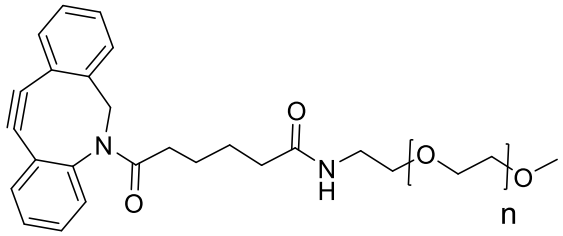
In a further example, the nonpeptidic, water-soluble polymer linked to the reactive group has the formula:

(RG)-(linker)-(POLY)-x

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 C_{1-20} alkyl; POLY is a nonpeptidic, water-soluble polymer; and x is an alcohol or methyl group at the terminus of the POLY.

In a further example, 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.

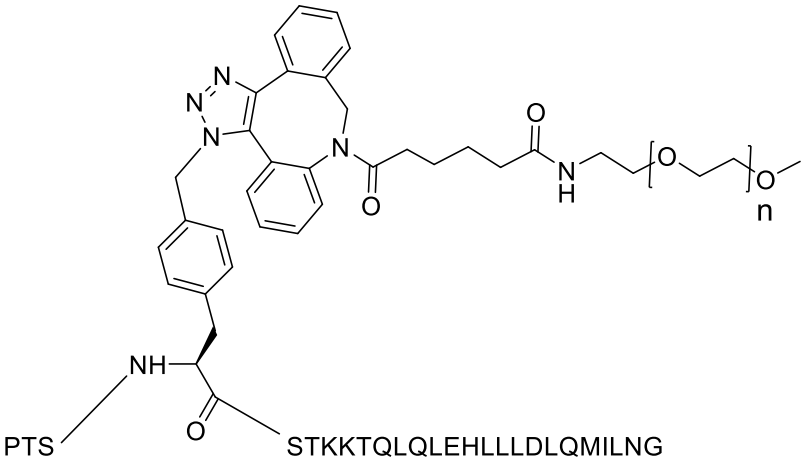
In a further example, the nonpeptidic, water-soluble polymer conjugated to the reactive group has the formula:



wherein n is about 681.

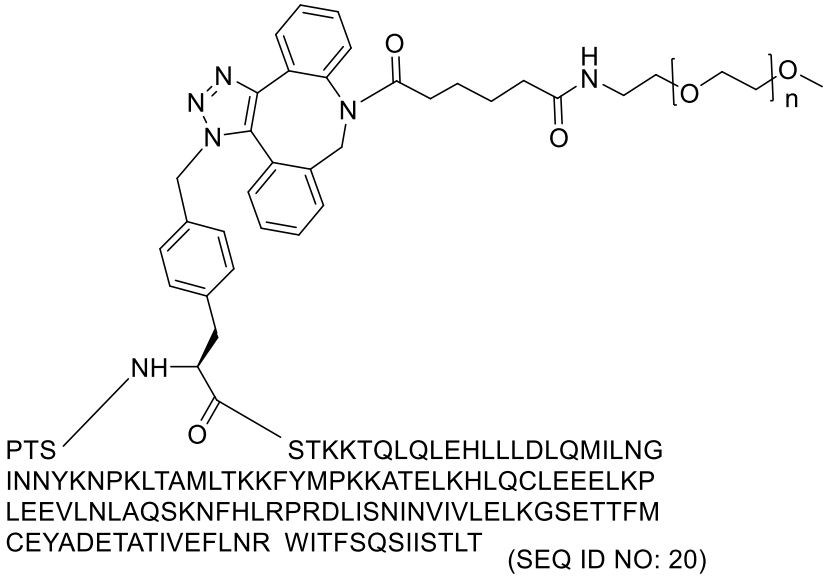
In a further example, the IL-2 conjugate comprises the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: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

In a further aspect, the present disclosure provides an interleukin 2 (IL-2) conjugate comprising the formula:



(SEQ ID NO: 20)

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



(SEQ ID NO: 20)

wherein n is about 681.

In a further aspect, the present disclosure provides a composition comprising: the IL-2 conjugate as described herein and a pharmaceutically acceptable carrier or excipient.

In a further example, the present disclosure provides a method for treating a proliferative disease or cancer in an individual, comprising: administering a therapeutically effective amount of any example of IL-2 conjugate disclosed herein or composition thereof to an individual in need thereof to treat the proliferative disease or cancer in the individual.

In a further aspect, the present disclosure provides the IL-2 conjugate as defined herein or the composition as defined herein for use in therapy.

In a further aspect, the present disclosure provides the IL-2 conjugate as defined herein or the composition as defined herein for use in combination with a therapeutic agent for use in therapy.

In a further example, the present disclosure provides a combination therapy for treating a proliferative disease or cancer in an individual, comprising: administering a therapeutically effective amount of any example of IL-2 conjugate disclosed herein or composition thereof 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. In a further example, the therapeutic agent is an anti-PD1 antibody or anti-PDL1 antibody. In a further example, the IL-2 conjugate

or composition is administered before the therapeutic agent is administered. In a further example, the IL-2 conjugate or composition is administered after the therapeutic agent is administered. In a further example, the IL-2 conjugate or composition is administered concurrently with the therapeutic agent.

The present disclosure further provides for the use of the IL-2 conjugate of any example of IL-2 conjugate disclosed herein or composition thereof for the treatment of a

proliferative disease or cancer. In a further example, is provided the use of the IL-2 conjugate of any example of IL-2 conjugate disclosed herein or composition thereof and a therapeutic agent for the treatment of a proliferative disease or cancer. In a further example, the therapeutic agent is an anti-PD1 antibody or anti-PDL1 antibody.

The present disclosure further provides for the use of the IL-2 conjugate of any example of IL-2 conjugate disclosed herein or composition thereof for the manufacture of a medicament for the treatment of a proliferative disease or cancer.

In a further aspect, the present disclosure provides use of the IL-2 conjugate as defined herein or the composition as defined herein in the manufacture of a medicament for the treatment of a proliferative disease or cancer.

In a further aspect, the present disclosure provides use of the IL-2 conjugate as defined herein or the composition as defined herein in the manufacture of a medicament for use with a therapeutic agent in a combination therapy for treatment of a proliferative disease or cancer.

The present disclosure further provides an IL-2 conjugate comprising an IL-2 polypeptide comprising an amino acid sequence with at least 80% 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.

In particular examples, the IL-2 polypeptide comprises an amino acid sequence with at least 85% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprises: (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.

In particular examples, the IL-2 polypeptide comprises an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprises: (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.

In particular examples, the IL-2 polypeptide comprises an amino acid sequence with at least 95% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprises: (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.

In a further example, the one or more amino acid substitutions in the IL-2 polypeptide are at positions R37 and F41. In a further example, the amino acid substitutions in the IL-2 polypeptide are R37A and F41K. In a further example, 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. In a further example, the IL-2 polypeptide further includes an N-terminal alanine residue.

In a further example, the non-natural amino acid is substituted for an amino acid corresponding to a position within the first 10 amino acids of the amino acid sequence as set forth in SEQ ID NO: 2 or inserted within said sequence. In a further example, the non-natural amino acid is substituted for an amino acid corresponding to a position within the sequence from P1 to Q10 of the amino acid sequence as set forth in SEQ ID NO: 2 or inserted within said sequence between P1 through Q10. In a further example, 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. In a further example, 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.

In a further example, the non-natural amino acid comprises a functional group and the nonpeptidic, water-soluble polymer is linked to a reactive group that is capable of reacting with the functional group to form a covalent linkage. In a further example, 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, phosphonoserine, phosphonotyrosine, 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)selanyl)propanoic acid, 2-amino-3-(phenylselanyl)propanoic, selenocysteine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, and p-propargyloxyphenylalanine. In a further example, the non-natural amino acid is p-azidomethyl-L-phenylalanine.

In a further example, the nonpeptidic, water-soluble polymer has an average molecular weight between 1 kDa and 100 kDa. In a further example, the nonpeptidic, water-soluble polymer has an average molecular weight of about 30 kDa. In a further example, 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(a-hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof. In a further example, the nonpeptidic, water-soluble polymer is a linear or branched PEG.

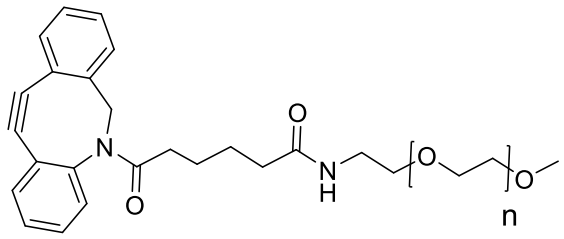
In a further example, the nonpeptidic, water-soluble polymer linked to the reactive group has the formula:

(RG)-(linker)-(POLY)-x

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 C_{1-20} alkyl; POLY is a nonpeptidic, water-soluble polymer; and x is an alcohol or methyl group at the terminus of the POLY.

In a further example, 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.

In a further example, the nonpeptidic, water-soluble polymer conjugated to the reactive group has the formula:



wherein n is about 681.

In a further example, 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.

The present disclosure further provides a composition comprising: the IL-2 conjugate of any example of IL-2 conjugate disclosed herein and a pharmaceutically acceptable carrier or excipient.

The present disclosure further provides 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 example of IL-2 conjugate disclosed herein or composition thereof to an individual in need thereof to treat the proliferative disease or cancer in the individual.

The present disclosure further provides 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 example of IL-2 conjugate disclosed herein or composition thereof 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. In a further example, the therapeutic agent is an anti-PD1 antibody or anti-PDL1 antibody. In a further example, the IL-2 conjugate or composition is administered before the therapeutic agent is administered. In a further example, the IL-2 conjugate or composition is administered after the therapeutic agent is administered. In a further example, the IL-2 conjugate or composition is administered concurrently with the therapeutic agent.

The present disclosure further provides for the use of the IL-2 conjugate of any example of IL-2 conjugate disclosed herein or composition thereof for the treatment of a proliferative disease or cancer.

The present disclosure further provides for the use of the IL-2 conjugate of any example of IL-2 conjugate disclosed herein or composition thereof and a therapeutic agent

for the treatment of a proliferative disease or cancer. In a further example, the therapeutic agent is an anti-PD1 antibody or anti-PDL1 antibody.

The present disclosure further provides for the use of the IL-2 conjugate of any example of IL-2 conjugate disclosed herein or composition thereof for the manufacture of a medicament for the treatment of a proliferative disease or cancer.

The present disclosure further provides 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. In a further example, the present disclosure provides a pharmaceutical composition comprising the IL-2 conjugate and a pharmaceutically acceptable carrier or excipient.

The present disclosure further provides 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.

The present disclosure further provides 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 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: 53.

In a further example, 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.

In a further example, the IL-2 polypeptide further includes an N-terminal alanine residue.

In a further example, the non-natural amino acid is substituted for an amino acid corresponding to a position within the first 10 amino acids of the amino acid sequence as set

forth in SEQ ID NO: 2 or inserted within said sequence. In a further example, the non-natural amino acid is substituted for an amino acid corresponding to a position within the sequence from P1 to Q10 of the amino acid sequence as set forth in SEQ ID NO: 2 or inserted within said sequence between P1 through Q10. In a further example, 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. In a further example, 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.

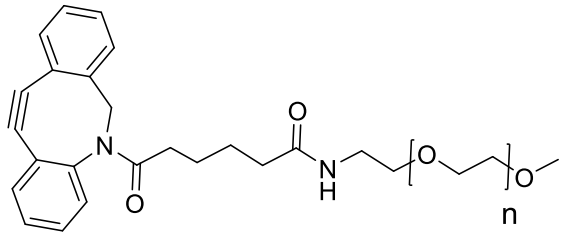
In a further example, the non-natural amino acid comprises a functional group and the water-soluble polymer is linked to a reactive group that is capable of reacting with the functional group to form a covalent linkage.

In a further example, 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, phosphonoserine, phosphonotyrosine, 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)selanyl)propanoic acid, 2-amino-3- (phenylselanyl)propanoic, selenocysteine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, and p-propargyloxyphenylalanine. In a further example, the non-natural amino acid is p-azidomethyl-L-phenylalanine.

In a further example, the nonpeptidic, water-soluble polymer has an average molecular weight between 1 kDa and 100 kDa. I n a further example, the nonpeptidic, water-soluble polymer has an average molecular weight of about 30 kDa. In a further example, The IL-2 conjugate of claim 65, 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(a-hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof. In a further example, the nonpeptidic, water-soluble polymer is a linear or branched PEG.

In a further example, 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.

In a further example, the nonpeptidic, water-soluble polymer, which comprises the alkyne, has the formula:



wherein n is about 681.

In a further example, the IL-2 conjugate comprises the amino acid sequence set forth in SEQ ID NO: 22.

The present disclosure further provides for any example disclosed herein in which the non-natural amino acid is not specifically identified as being substituted for the serine residue at position four as it corresponds to position four of SEQ ID NO:2, examples wherein the substitution or insertion of the non-natural amino acid is within the N-terminal region, for example, (i) a substitution of an amino acid within the first 30 amino acids of 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 within the first 30 amino acids of the N-terminus of the IL-2 polypeptide or (ii) a substitution of an amino acid within the first 20 amino acids of 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 within the first 20 amino acids of the N-terminus of the IL-2 polypeptide.

Thus, the present disclosure provides (a) 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 that reduce(s) affinity of the IL-2 polypeptide for the human IL-2R$\alpha\beta\gamma_C$ relative to wild-type human IL-2; and (ii) a substitution of an amino acid within the N-terminal region 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 within the N-terminal region of the IL-2 polypeptide;

wherein the IL-2 polypeptide has substantially similar binding affinity for the human IL-2R$\beta\gamma_C$ relative to wild-type human IL-2.

In particular examples, the present disclosure provides (a) 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 that reduce(s) affinity of the IL-2 polypeptide for the human IL-2R$\alpha\beta\gamma_C$ relative to wild-type human IL-2; and (ii) a substitution of an amino acid within the first 30 amino acids of 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 within the first 30 amino acids of the N-terminus of the IL-2 polypeptide; wherein the IL-2 polypeptide has substantially similar binding affinity for the human IL-2R$\beta\gamma_C$ relative to wild-type human IL-2; and (b) 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 that reduce(s) affinity of the IL-2 polypeptide for the human IL-2R$\alpha\beta\gamma_C$ relative to wild-type human IL-2; and (ii) a substitution of an amino acid within the first 20 amino acids of 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 within the first 20 amino acids of the N-terminus of the IL-2 polypeptide; wherein the IL-2 polypeptide has substantially similar binding affinity for the human IL-2R$\beta\gamma_C$ relative to wild-type human IL-2.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the interaction of IL-2 with the low affinity (α), intermediate affinity ($\beta\gamma_C$), and high affinity ($\alpha\beta\gamma_C$) IL-2 receptors. IL-2 binds the α monomer with low affinity (no signal transduction). IL-2 binds the $\beta\gamma_c$ dimer with intermediate affinity. The $\beta\gamma_c$ dimer is expressed on $CD8^+$ T cells and NK cells. IL-2 binds the $\alpha\beta\gamma_c$ trimer with high affinity. The $\alpha\beta\gamma_c$ trimer is expressed on T_{regs} and activated T cells. High doses of IL-2 activate the $\beta\gamma_c$ dimer, which activates the immune response. However, high doses also activate the $\alpha\beta\gamma_c$ trimer on T_{regs}, which suppresses activation of the immune response and may lead to tolerance of tumor antigens.

WHAT IS CLAIMED:

1. An interleukin 2 (IL-2) conjugate comprising an IL-2 polypeptide comprising an amino acid sequence with at least 80% identity to the amino acid sequence set forth in SEQ ID NO: 53 and further comprising:

(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 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.

2. The IL-2 conjugate of claim 1, wherein 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 according to numbering scheme B.

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-2Rα) as determined by a Surface Plasmon Resonance assay.

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 according to numbering scheme B.

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 or N-terminal methionine 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, -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, phosphonoserine, phosphonotyrosine, 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)selanyl)propanoic acid,

2-amino-3- (phenylselanyl)propanoic, selenocysteine, m-acetylphenylalanine, 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(a-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:

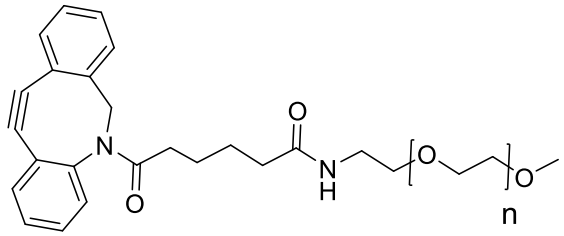
(RG)-(linker)-(POLY)-x

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 C_{1-20} 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.

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

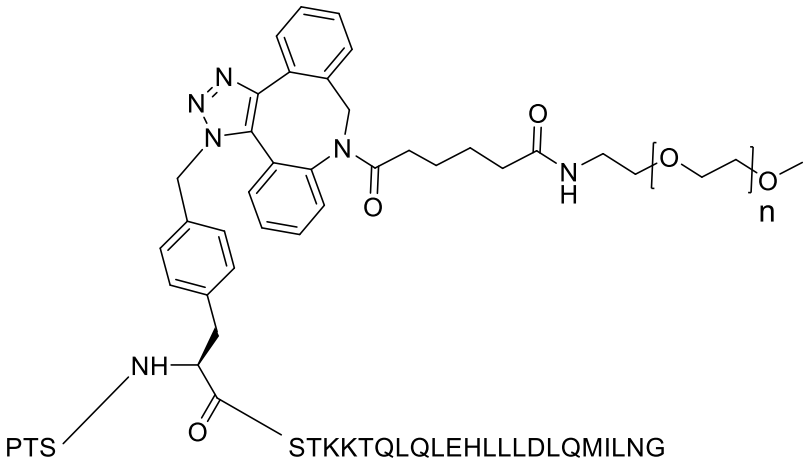


wherein n is about 681.

21. The IL-2 conjugate of claim 1, wherein the IL-2 polypeptide comprises an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO: 53.

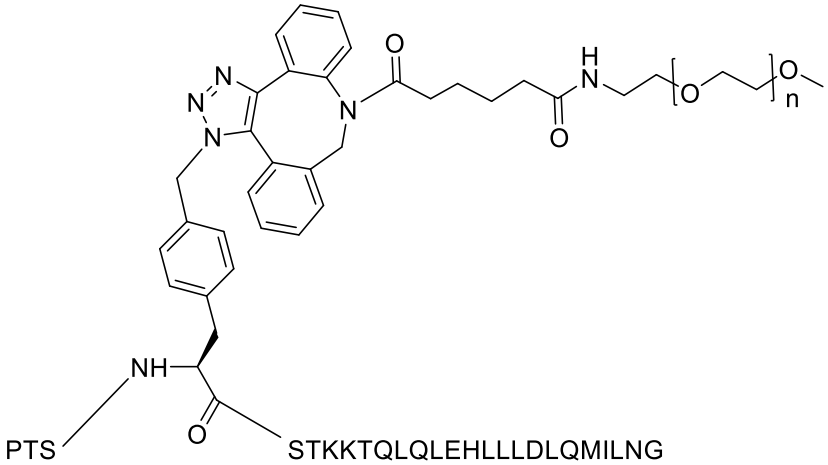
22. The IL-2 conjugate of claim 1, wherein the IL-2 polypeptide comprises an amino acid sequence with at least 95% identity to the amino acid sequence set forth in SEQ ID NO: 53.

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



(SEQ ID NO: 20)

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



(SEQ ID NO: 20)

wherein n is about 681.

24. The interleukin 2 (IL-2) conjugate of claim 23, wherein the IL-2 polypeptide further includes an N-terminal methionine residue.

25. The interleukin 2 (IL-2) conjugate of claim 23, wherein the IL-2 polypeptide further includes an N-terminal alanine residue.

26. A composition comprising:

the IL-2 conjugate of any one of claims 1 to 22 and a pharmaceutically acceptable carrier or excipient.

27. A composition comprising:

the IL-2 conjugate of any one of claims 23 to 25 and a pharmaceutically acceptable carrier or excipient.

28. The IL-2 conjugate of any one of claims 1-25 or the composition of claim 26 or 27 for use in therapy.

29. The IL-2 conjugate of any one of claims 1-25 or the composition of claim 26 or 27 for use in combination with a therapeutic agent for use in therapy.

30. Use of the IL-2 conjugate of any one of claims 1-25 or the composition of claim 26 or 27 in the manufacture of a medicament for the treatment of a proliferative disease or cancer.

31. Use of the IL-2 conjugate of any one of claims 1-25 or the composition of claim 26 or 27 in the manufacture of a medicament for use with a therapeutic agent in a combination therapy for 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.