## CLAIMS

1. An IL-2 conjugate comprising: an IL-2 polypeptide comprising a substituted lysine comprising an azidoethoxy moiety covalently attached to a conjugating moiety comprising a polyethylene glycol (PEG), wherein:

the IL-2 polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 1; and

the substituted lysine substitutes for an amino acid acid residue within the IL-2 polypeptide at position K35, F42, K43, E62, P65, R38, T41, E68, Y45, or V69 in reference to the amino acid positions within SEQ ID NO: 1.

2. The IL-2 conjugate of claim 1, wherein the PEG has a molecular weight of about 5 kDa, about 10 kDa, about 15 kDa, about 20 kDa, about 25 kDa, about 30 kDa, about 35 kDa, about 40 kDa, about 45 kDa, or about 50 kDa.

3. The IL-2 conjugate of claim 1 or 2, wherein the substituted lysine substitutes for the amino acid at position P65 in reference to the amino acid positions within SEQ ID NO: 1.

4. The IL-2 conjugate of any one of claims 1-3, wherein the IL-2 polypeptide comprises an N-terminal deletion of one residue relative to SEQ ID NO: 1.

5. The IL-2 conjugate of any one of claims 1-4, wherein the PEG has a molecular weight of about 30 kDa.

6. The IL-2 conjugate of any one of claims 1-5, wherein the substituted lysine is *N*6-(2-azidoethoxy)-carbonyl-lysine.

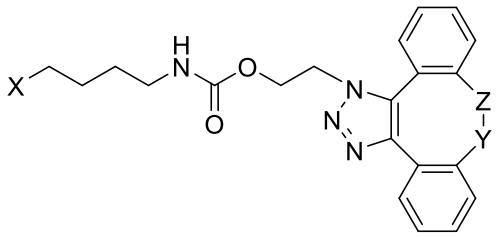
7. The IL-2 conjugate of any one of claims 1-6, wherein the substituted lysine is *N*6-((2-azidoethoxy)-carbonyl)-L-lysine.

8. The IL-2 conjugate of any one of claims 1-7, wherein the IL-2 polypeptide comprises an amino acid sequence having about 95% sequence identity to SEQ ID NO: 1.

9. The IL-2 conjugate of any one of claims 1-8, wherein the IL-2 polypeptide comprises an amino acid sequence having about 97% sequence identity to SEQ ID NO: 1.

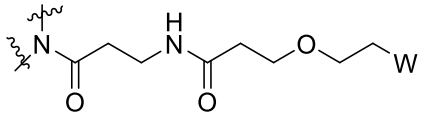
10. The IL-2 conjugate of any one of claims 1-9, wherein the IL-2 polypeptide comprises an amino acid sequence having about 98% sequence identity to SEQ ID NO: 1.

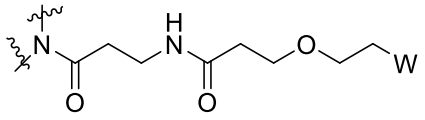
11. An IL-2 conjugate comprising an IL-2 polypeptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 3 in which at least one amino acid residue at an amino acid position selected from K34, F41, K42, E61, P64, R37, T40, E67, Y44, and V68 in reference to the amino acid positions within SEQ ID NO: 3 is replaced by the structure of Formula (I):

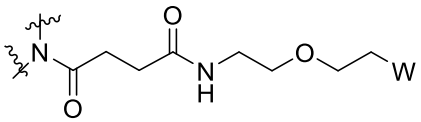


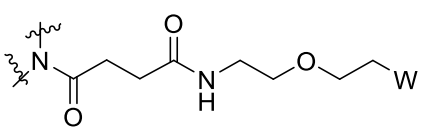
(I)

wherein:

Z is $CH_2$ and Y is  ;

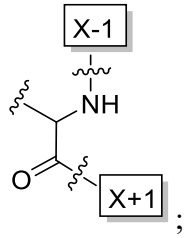
Y is $CH_2$ and Z is  ;

Z is $CH_2$ and Y is  ; or

Y is $CH_2$ and Z is  ;

W is a PEG group having a molecular weight of about 5 kDa, about 10 kDa, about 15 kDa, about 20 kDa, about 25 kDa, about 30 kDa, about 35 kDa, about 40 kDa, about 45 kDa, about 50 kDa, or about 60 kDa;

X has the structure:



;

X-1 indicates the point of attachment to the amino acid residue preceding X; and

X+1 indicates the point of attachment to the amino acid residue following X.

12. The IL-2 conjugate of claim 11, wherein the IL-2 polypeptide comprises an amino acid sequence having about 95% sequence identity to SEQ ID NO: 3.

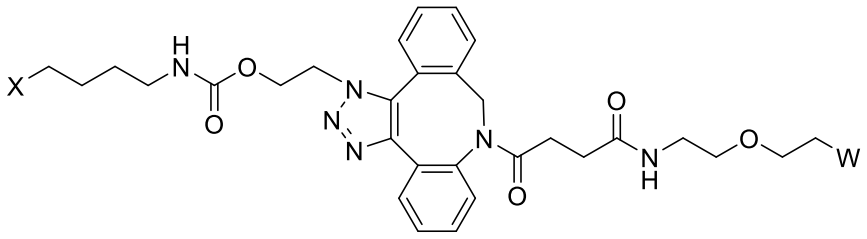
13. The IL-2 conjugate of claim 11 or 12, wherein the IL-2 polypeptide comprises an amino acid sequence having about 97% sequence identity to SEQ ID NO: 3.

14. The IL-2 conjugate of any one of claims 11-13, wherein the IL-2 polypeptide comprises an amino acid sequence having about 98% sequence identity to SEQ ID NO: 3.

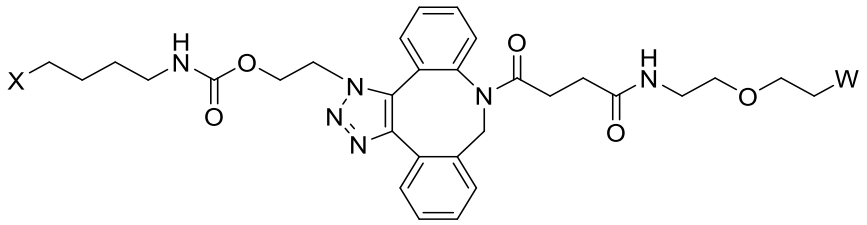
15. The IL-2 conjugate of any one of claims 11-14, wherein the IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO: 3 in which at least one amino acid residue at an amino acid position selected from K34, F41, K42, E61, P64, R37, T40, E67, Y44, and V68 in reference to the amino acid positions within SEQ ID NO: 3 is replaced by the structure of Formula (I).

16. The IL-2 conjugate of any one of claims 11-15, wherein the at least one amino acid residue at position P64 in reference to the amino acid position within SEQ ID NO: 3 is replaced by the structure of Formula (I).

17. The IL-2 conjugate of any one of claims 11-16, wherein the structure of Formula (I) has the structure of Formula (IV) or Formula (V):



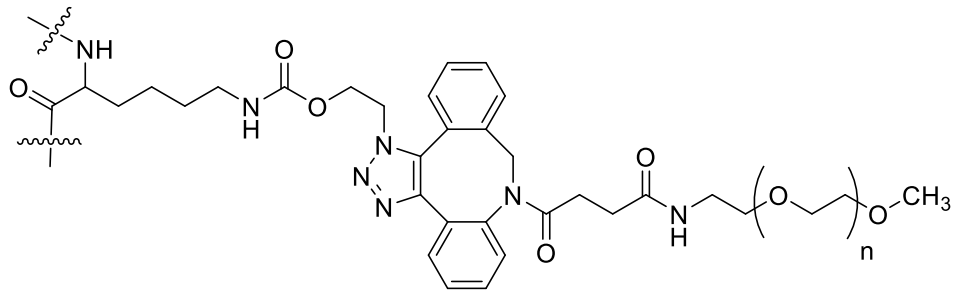
(IV)



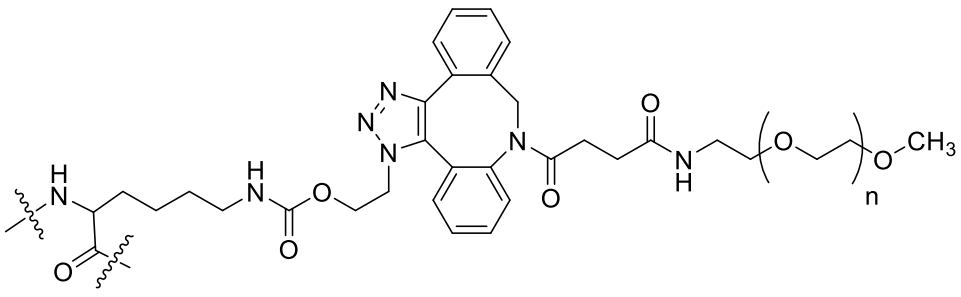
(V) .

18. The IL-2 conjugate of any one of claims 11-17, wherein W is a PEG group having a molecular weight of about 30 kDa.

19. The IL-2 conjugate of any one of claims 11-15, wherein the at least one amino acid residue at position P64 in reference to the amino acid position within SEQ ID NO: 3 is replaced by the structure of Formula (XII) or Formula (XIII):



(XII)



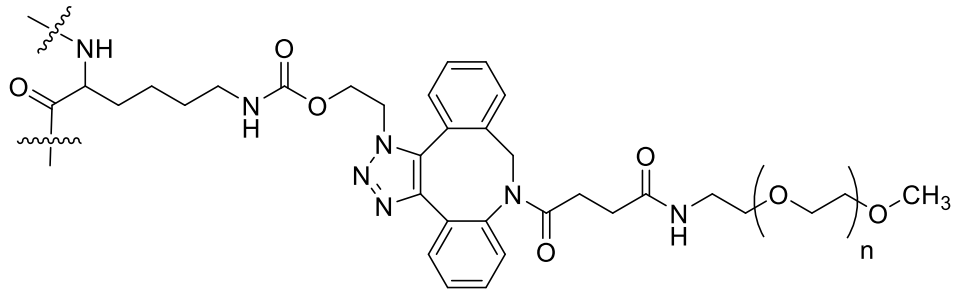
(XIII)

wherein:

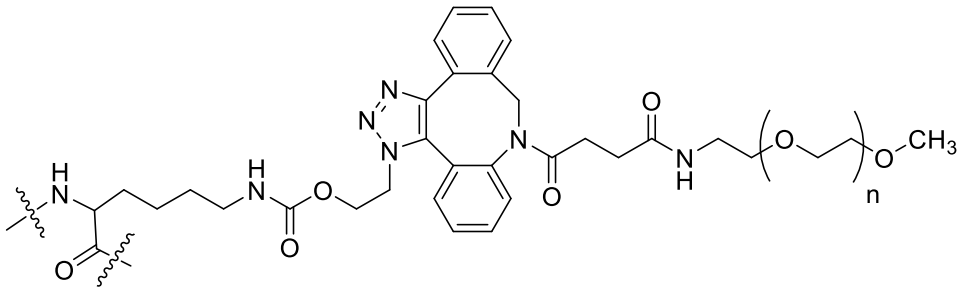
n is an integer such that a PEG group having the structure of -$(OCH_2CH_2)_n$-$OCH_3$ has a molecular weight of about 30 kDa; and

the wavy lines indicate covalent bonds to the first amino acid residue preceding the at least one amino acid at position P64 and the first amino acid residue following the at least one amino acid residue at position P64, respectively, within SEQ ID NO: 3.

20. The IL-2 conjugate of any one of claims 11-15, wherein the at least one amino acid residue at position P64 in reference to the amino acid position within SEQ ID NO: 3 is replaced by the structure of Formula (XII) or Formula (XIII):



(XII)



(XIII)

wherein:

n is an integer such that a PEG group having the structure of -$(OCH_2CH_2)$n-$OCH_3$ has a molecular weight of about 5 kDa, about 10 kDa, about 15 kDa, about 20 kDa, about 25 kDa, about 30 kDa, about 35 kDa, about 40 kDa, about 45 kDa, about 50 kDa, or about 60 kDa; and

the wavy lines indicate covalent bonds to the first amino acid residue preceding the at least one amino acid at position P64 and the first amino acid residue following the at least one amino acid residue at position P64, respectively, within SEQ ID NO: 3.

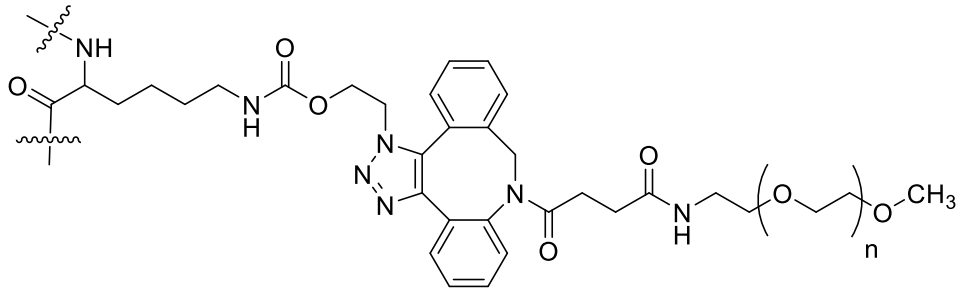
21. The IL-2 conjugate of any one of claims 1-20, which is a pharmaceutically acceptable salt, solvate, or hydrate.

22. A pharmaceutical composition comprising the IL-2 conjugate of any one of claims 1-21 and a pharmaceutically acceptable excipient.

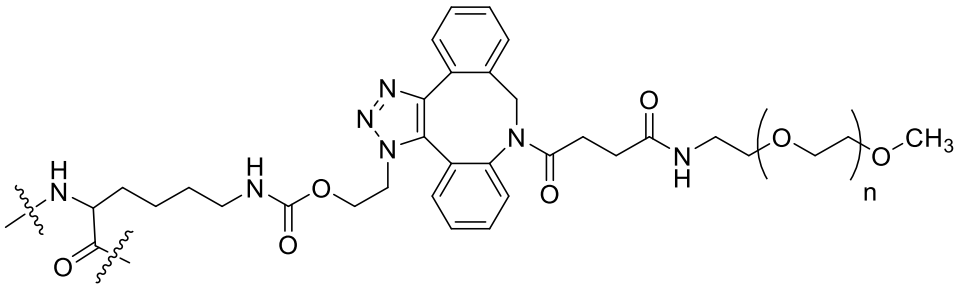
23. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 17 or 18, wherein the mixture comprises (i) an IL-2 conjugate in which the structure of Formula (I) has the structure of Formula (IV) and (ii) an IL-2 conjugate in which the structure of Formula (I) has the structure of Formula (V).

24. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 19 or 20, wherein the mixture comprises (i) an IL-2 conjugate in which the amino acid residue at position P64 in reference to the amino acid position within SEQ ID NO: 3 is replaced by the structure of Formula (XII) and (ii) an IL-2 conjugate in which the amino acid residue at position P64 in reference to the amino acid position within SEQ ID NO: 3 is replaced by the structure of Formula (XIII).

25. An IL-2 conjugate consisting of the amino acid sequence of SEQ ID NO: 3 in which a modified amino acid of the structure of Formula (XII) or Formula (XIII) is present at position 64 of SEQ ID NO: 3 instead of proline:




(XII)



(XIII)

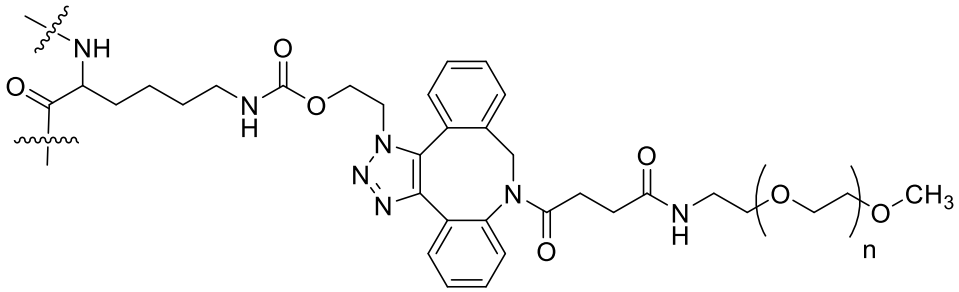
wherein:

n is an integer such that a PEG group having the structure of -$(OCH_2CH_2)_n$-$OCH_3$ has a molecular weight of about 30 kDa; and

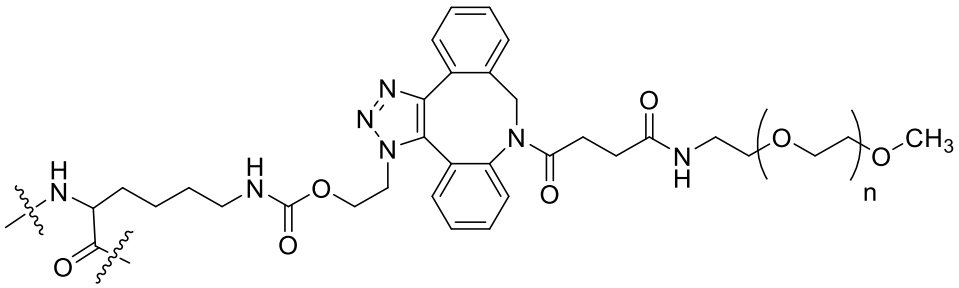
the wavy lines indicate covalent bonds to an amino acid residue at position 63 and an amino acid residue at position 65, respectively, within SEQ ID NO: 3.

26. An IL-2 conjugate comprising the amino acid sequence of SEQ ID NO: 30, wherein X in SEQ ID NO: 30 is N6-(2-azidoethoxy)-carbonyl-L-lysine (AzK) covalently attached via a conjugating moiety comprising a dibenzocyclooctyne group to a PEG group having a molecular weight of about 30 kDa.

27. The IL-2 conjugate of claim 26, wherein the AzK covalently attached via the conjugating moiety to the PEG group is collectively represented by the structure of Formula (XII) or Formula (XIII):



(XII)



(XIII)

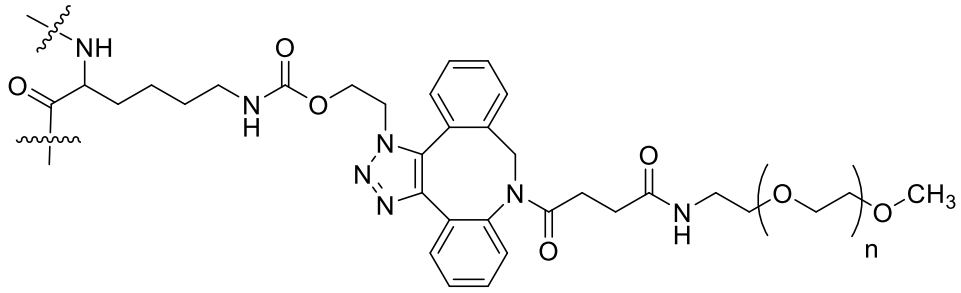
wherein:

n is an integer such that the PEG group having the structure of -$(OCH_2CH_2)_n$-$OCH_3$ has a molecular weight of about 30 kDa; and

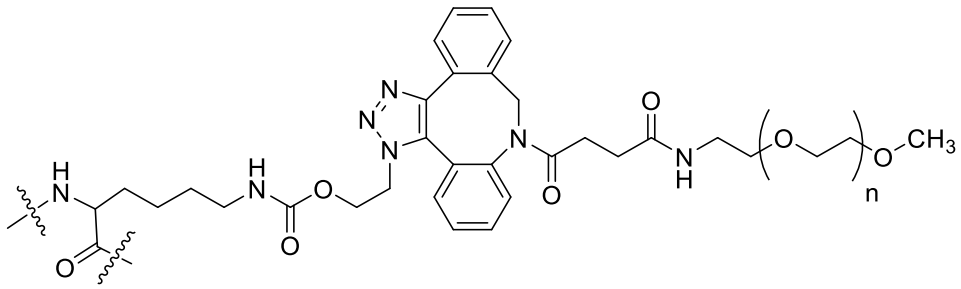
the wavy lines indicate covalent bonds to the first amino acid residue preceding the X and the first amino acid residue following the X, respectively, within SEQ ID NO: 30.

28. An IL-2 conjugate comprising an amino acid sequence that (i) is at least 98% identical to the amino acid sequence of SEQ ID NO: 30 and (ii) includes X at a position corresponding to position 64 of SEQ ID NO: 30, wherein X is N6-(2-azidoethoxy)-carbonyl-L-lysine (AzK) covalently attached via a conjugating moiety comprising a dibenzocyclooctyne group to a PEG group having a molecular weight of about 30 kDa.

29. The IL-2 conjugate of claim 28, wherein the AzK covalently attached via the conjugating moiety to the PEG group is collectively represented by the structure of Formula (XII) or Formula (XIII):



(XII)



(XIII)

wherein:

n is an integer such that the PEG group having the structure of -$(OCH_2CH_2)_n$-$OCH_3$ has a molecular weight of about 30 kDa; and

the wavy lines indicate covalent bonds to the first amino acid residue preceding the X and the first amino acid residue following the X, respectively, within SEQ ID NO: 30.

30. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 27 or 29, wherein the mixture comprises (i) an IL-2 conjugate comprising the structure of Formula (XII), and (ii) an IL-2 conjugate comprising the structure of Formula (XIII).

31. The pharmaceutical composition of claim 30, further comprising a diluent, a buffering agent, a stabilizer, a surfactant, or any combination thereof.

32. The pharmaceutical composition of claim 30, further comprising sorbitol.

33. The pharmaceutical composition of claim 30, further comprising polysorbate-20.

34. A dispenser device comprising the pharmaceutical composition of any one of claims 22-24 and 30-33.

35. A syringe comprising the pharmaceutical composition of any one of claims 22-24 and 30-33.

36. A vial comprising the pharmaceutical composition of any one of claims 22-24 and 30-33.

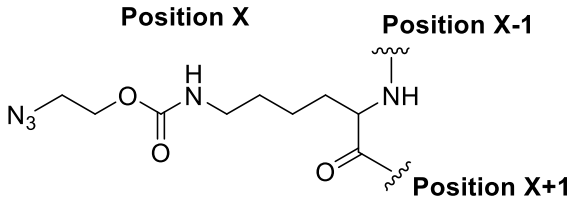
37. Use of the IL-2 conjugate of any one of claims 1-21 and 25-29 or the pharmaceutical composition of any one of claims 22-24 and 30-33 in the manufacture of a medicament for treating cancer in a subject in need thereof.

38. The use of claim 37, wherein the cancer is selected from renal cell carcinoma (RCC), melanoma, non-small cell lung cancer (NSCLC), head and neck squamous cell cancer (HNSCC), urothelial carcinoma (UC), Merkel cell carcinoma (MCC), ovarian cancer, gastric cancer, breast cancer, bladder cancer, bone cancer, brain cancer, colorectal cancer, esophageal cancer, eye cancer, head and neck cancer, kidney cancer, lung cancer, pancreatic cancer, prostate cancer, cutaneous squamous cell carcinoma (CSCC), and classical Hodgkin lymphoma (cHL).

39. The use of claim 37 or 38, wherein the medicament is to be administered in combination with an additional therapeutic agent.

40. Use of the IL-2 conjugate of any one of claims 1-21 and 25-29 or the pharmaceutical composition of any one of claims 22-24 and 30-33 in the manufacture of a medicament for selectively expanding CD4+ helper T cells, CD8+ effector naïve T cells, CD8+ effector memory T cells, NK cells, NKT cells, or a combination thereof in a subject in need thereof.

41. A method of making an IL-2 conjugate, comprising:
reacting an IL-2 polypeptide, or a pharmaceutically acceptable salt, solvate, or hydrate thereof, comprising an unnatural amino acid having the structure:



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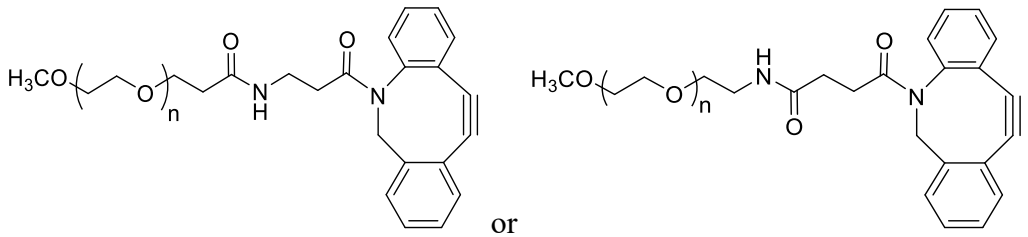
wherein the IL-2 polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 1, wherein Position X indicates the position of the amino acid residue within SEQ ID NO: 1 for which the unnatural amino acid substitutes, wherein Position X is K35, F42, K43, E62, P65, R38, T41, E68, Y45, or V69 in reference to the amino acid positions within SEQ ID NO: 1, Position X-1 indicates position of the amino acid residue within SEQ ID NO: 1 preceding the unnatural amino acid, Position X+1 indicates the position of the amino acid residue within SEQ ID NO: 1 following the unnatural amino acid, and
wherein the wavy lines indicate covalent bonds to amino acid residues at Position X-1 and Position X+1, respectively, within SEQ ID NO: 1,
with an mPEG-DBCO of formula


or

wherein n is an integer such that the mPEG-DBCO comprises an mPEG having a molecular weight of about 5 kDa, about 10 kDa, about 15 kDa, about 20 kDa, about 25 kDa, about 30 kDa, about 35 kDa, about 40 kDa, about 45 kDa, about 50 kDa, or about 60 kDa,

to form an IL-2 conjugate.

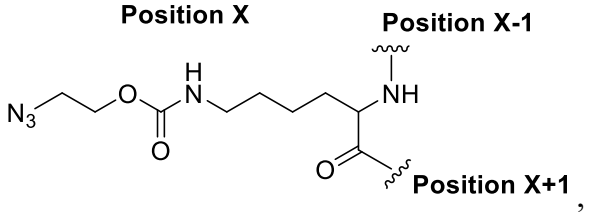
42. The method of claim 41, wherein Position X is P65 in reference to the amino acid position within SEQ ID NO: 1.

43. The method of claim 41 or 42, wherein the IL-2 polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 1.

44. The method of any one of claims 41-43, wherein the IL-2 polypeptide comprises an amino acid sequence having at least 97% sequence identity to SEQ ID NO: 1.

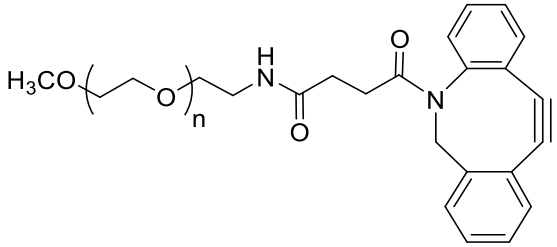
44. The method of any one of claims 41-44, wherein the IL-2 polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 1.

45. A method of making an IL-2 conjugate, comprising:
reacting an IL-2 polypeptide, or a pharmaceutically acceptable salt, solvate, or hydrate thereof, comprising an unnatural amino acid having the structure:



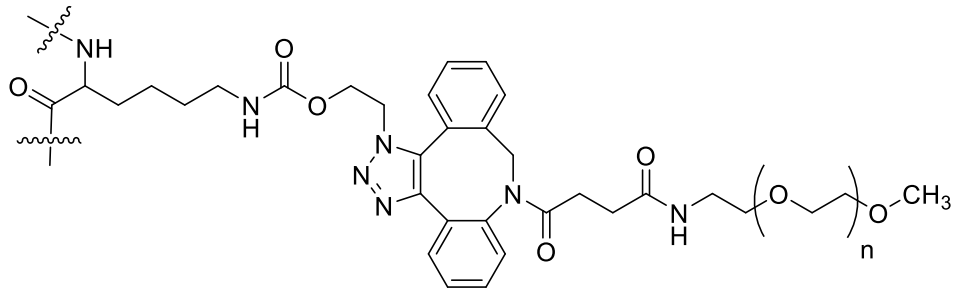
,

wherein the IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO: 3 in which the unnatural amino acid substitutes for an amino acid residue within SEQ ID NO: 3 at Position X which is at P64 in reference to the amino acid position within SEQ ID NO: 3, wherein Position X-1 indicates the position of the amino acid residue within SEQ ID NO: 3 preceding the unnatural amino acid, Position X+1 indicates the position of the amino acid residue within SEQ ID NO: 3 following the unnatural amino acid,
with an mPEG-DBCO of formula

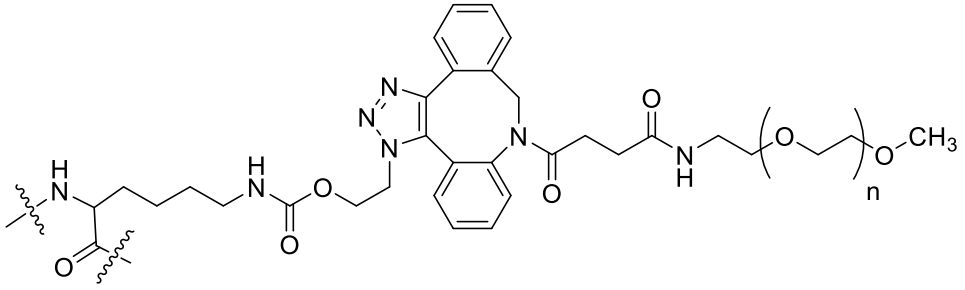



wherein n is an integer such that the mPEG-DBCO comprises an mPEG having a molecular weight of about 30 kDa,

to form an IL-2 conjugate comprising an unnatural amino acid at P64 (Position X) in reference to the amino acid position within SEQ ID NO: 3, wherein the unnatural amino acid is covalently attached to the mPEG, wherein the unnatural amino acid covalently attached to the mPEG, collectively, has the structure of Formula (XII) or Formula (XIII):



(XII)



(XIII)

wherein:

the wavy lines indicate covalent bonds to amino acid residues at Position X-1 and Position X+1, respectively, within SEQ ID NO: 3.