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 ~~80~~90% sequence identity to SEQ ID NO: 1; and

the substituted lysine substitutes for an amino acid at position K35, F42, ~~F44,~~ K43, E62, P65, R38, T41, E68, Y45, or V69~~, or L72~~ in reference to the amino acid positions within SEQ ID NO: 1.

2. The IL-2 conjugate of claim 1, which is a pharmaceutically acceptable salt, solvate, or hydrate.

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

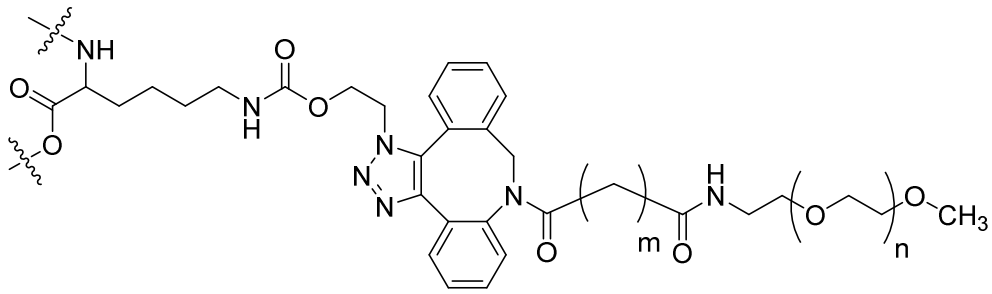
4. The IL-2 conjugate of claim 1, wherein the substituted lysine substitutes for the amino acid at position P65 in reference to the amino acid positions within SEQ ID NO: 1.

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

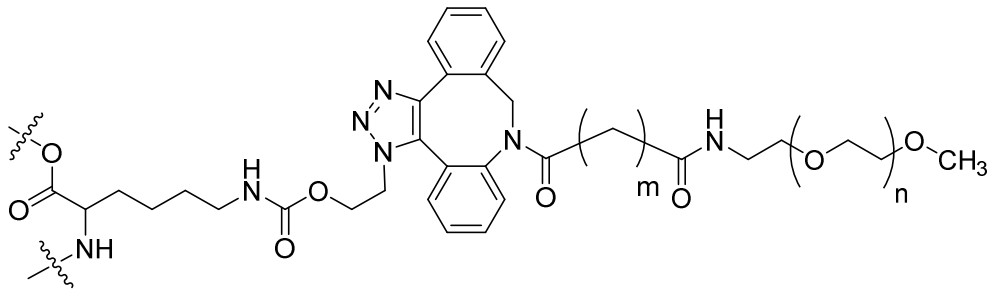
6. The IL-2 conjugate of claim 4, wherein the PEG has a molecular weight of about 30 kDa.

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

8. An IL-2 conjugate comprising the amino acid sequence of SEQ ID NO: 50, wherein [AzK_L1_PEG30kD] is an L-amino acid having the structure of Formula (XVI) or Formula (XVII):



~~Formula~~ (XVI~~);~~)



~~Formula~~ (XVII~~);~~)

wherein:

m is 2;

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

the wavy lines indicate covalent bonds to amino acid residues within SEQ ID NO: 50 that are not replaced.

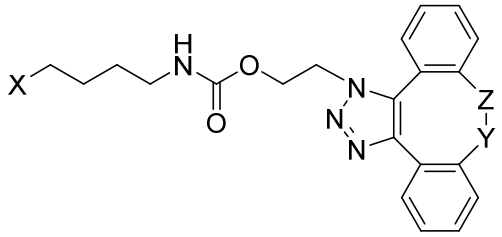
9. The IL-2 conjugate of claim 8, which is a pharmaceutically acceptable salt, solvate, or hydrate.

10. A pharmaceutical composition comprising the IL-2 conjugate of claim 1 and a pharmaceutically acceptable excipient.

11. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 8 and a pharmaceutically acceptable excipient, wherein the mixture comprises IL-2 conjugates in which the [AzK_L1_PEG30kD] is an L-amino acid having the structure of Formula (XVI) and

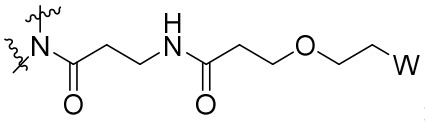
IL-2 conjugates in which the [AzK_L1_PEG30kD] is an L-amino acid having the structure of Formula (XVII).

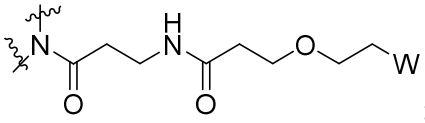
12. An IL-2 conjugate comprising the amino acid sequence of SEQ ID NO: 3, wherein at least one amino acid at position K34, F41, ~~F43,~~ K42, E61, P64, R37, T40, E67, Y44, or V68~~, or L71~~ is replaced by the structure of Formula (I):

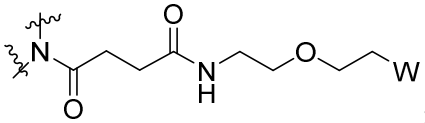


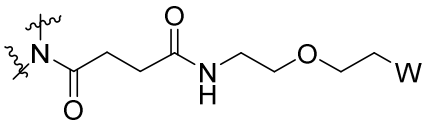
~~Formula~~ (I)

wherein:

Z is CH_2 and Y is  ;

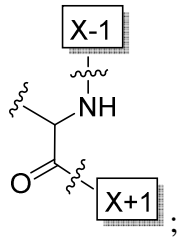
Y is CH_2 and Z is  ;

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, 10 kDa, 15 kDa, 20 kDa, 25 kDa, 30 kDa, 35 kDa, 40 kDa, 45 kDa, 50 kDa, or 60 kDa;

X is an L-amino acid having the structure:



;

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

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

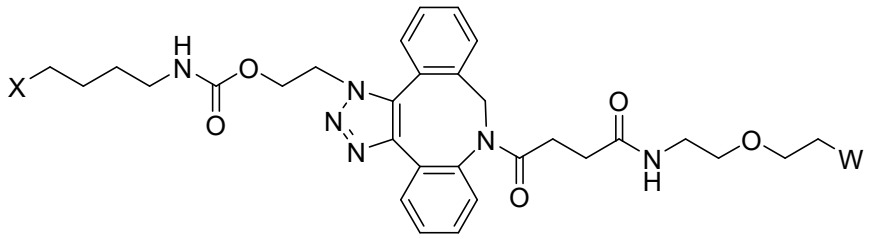
13. The IL-2 conjugate of claim 12, which is a pharmaceutically acceptable salt, solvate, or hydrate.

14. The IL-2 conjugate of claim 12, wherein the PEG group has a molecular weight of about 30 kDa.

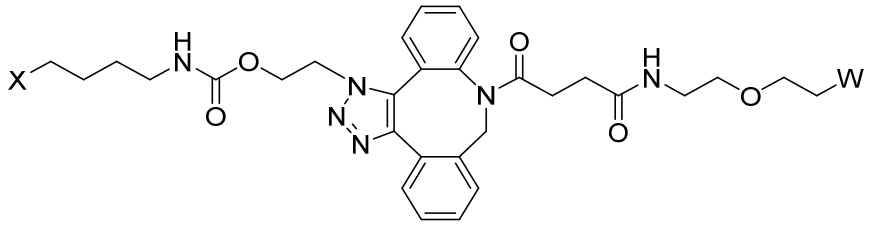
15. The IL-2 conjugate of claim 12, wherein the amino acid at position P64 is replaced by the structure of Formula (I).

16. The IL-2 conjugate of claim 15, wherein the PEG group has a molecular weight of about 30 kDa.

17. The IL-2 conjugate of claim 12, wherein the structure of Formula (I) has the structure of Formula (IV) or Formula (V):



~~Formula~~ (IV~~);~~)



~~Formula~~ (V~~);~~)

wherein:

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

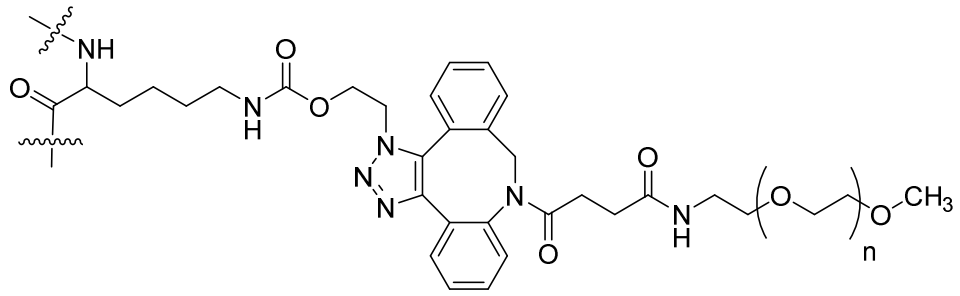
18. The IL-2 conjugate of claim 17, wherein W is a PEG group having a molecular weight of about 30 kDa.

19. A pharmaceutical composition comprising the IL-2 conjugate of claim 12 and a pharmaceutically acceptable excipient.

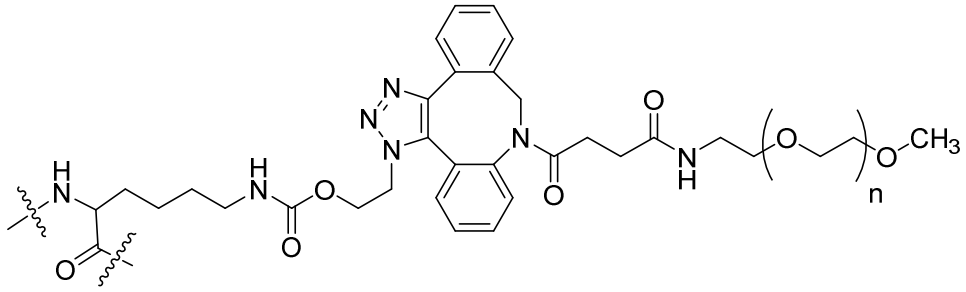
20. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 17 and a pharmaceutically acceptable excipient, wherein the mixture comprises IL-2 conjugates in which the structure of Formula (I) has the structure of Formula (IV) and IL-2 conjugates in which the structure of Formula (I) has the structure of Formula (V).

21. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 18 and a pharmaceutically acceptable excipient, wherein the mixture comprises IL-2 conjugates in which the structure of Formula (I) has the structure of Formula (IV) and IL-2 conjugates in which the structure of Formula (I) has the structure of Formula (V).

22. The IL-2 conjugate of claim 12, wherein amino acid P64 is replaced by the structure of Formula (XII) or Formula (XIII):



~~Formula~~ (XII~~);~~)



~~Formula~~ (XIII~~);~~)

wherein:

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

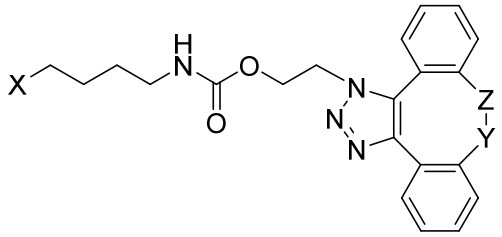
the wavy lines indicate covalent bonds to amino acid residues within SEQ ID NO: 3 that are not replaced.

23. The IL-2 conjugate of claim 22, which is a pharmaceutically acceptable salt, solvate, or hydrate.

24. A pharmaceutical composition comprising the IL-2 conjugate of claim 22 and a pharmaceutically acceptable excipient.

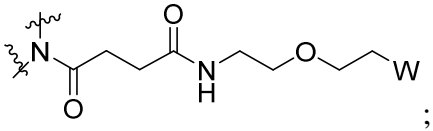
25. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 22 and a pharmaceutically acceptable excipient, wherein the mixture comprises IL-2 conjugates in which amino acid P64 is replaced by the structure of Formula (XII) and IL-2 conjugates in which amino acid P64 is replaced by the structure of Formula (XIII).

26. An IL-2 conjugate comprising the amino acid sequence of SEQ ID NO: 3, wherein the amino acid at position P64 is replaced by the structure of Formula (I):

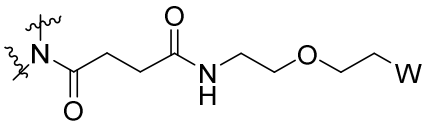


~~Formula~~ (I)

wherein:

Z is CH_2 and Y is 

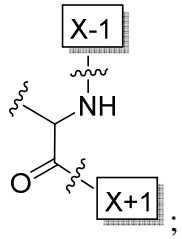
; or

Y is CH_2 and Z is 

;

W is a PEG group having a molecular weight of about 30 kDa;

X is an L-amino acid having the structure:



;

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

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

27. The IL-2 conjugate of claim 26, which is a pharmaceutically acceptable salt, solvate, or hydrate.

28. The IL-2 conjugate of claim 1, wherein the PEG has a molecular weight of about 30 kDa.

29. The IL-2 conjugate of claim 1, wherein the IL-2 comprises an amino acid sequence having about 95% sequence identity to SEQ ID NO: 1.

30. The IL-2 conjugate of claim 29, wherein the IL-2 comprises an amino acid sequence having about 97% sequence identity to SEQ ID NO: 1.

31. The IL-2 conjugate of claim 1, wherein the IL-2 comprises an amino acid sequence having about 99% sequence identity to SEQ ID NO: 50.

32. The pharmaceutical composition of claim 24, wherein the average molecular weight of $-(OCH_2CH_2)_n-OCH_3$ in the IL-2 conjugates in the pharmaceutical composition is about 30 kDa.

33. The pharmaceutical composition of claim 25, wherein the average molecular weight of $-(OCH_2CH_2)_n-OCH_3$ in the IL-2 conjugates in the pharmaceutical composition is about 30 kDa.