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% 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, 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.

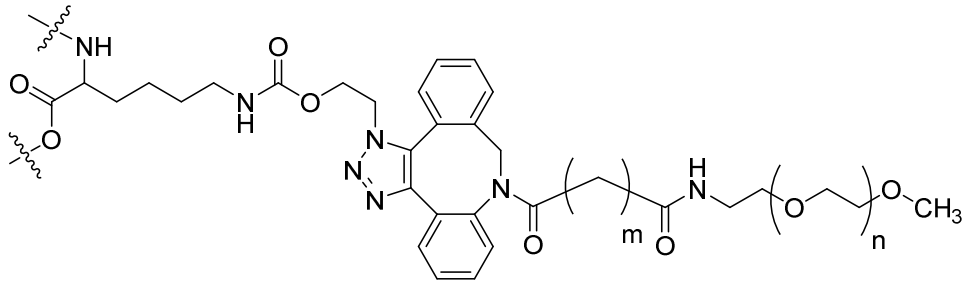
4. The IL-2 conjugate of claim 1, wherein the substituted lysine substitutes for the amino acid at position P65 in reference to the amino acid positions within SEQ ID NO: 1.

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.

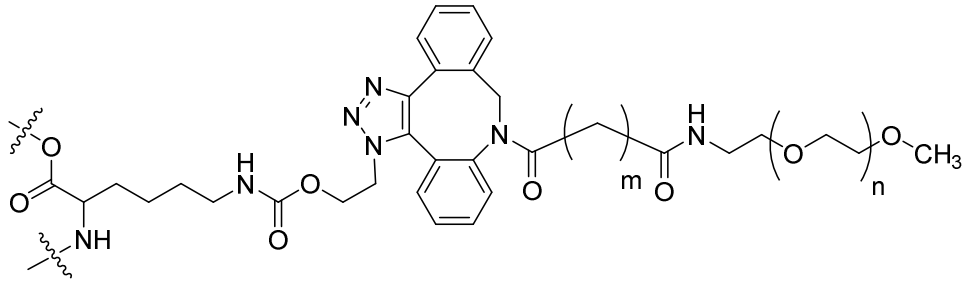
6. The IL-2 conjugate of claim 4, wherein the PEG has a molecular weight of about 30 kDa.

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.

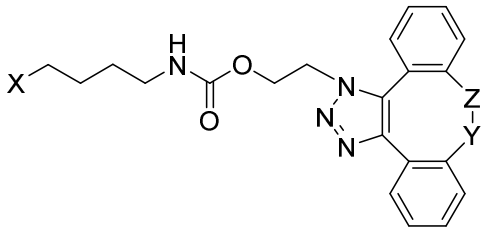
9. The IL-2 conjugate of claim 8, which is a pharmaceutically acceptable salt, solvate, or hydrate.

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

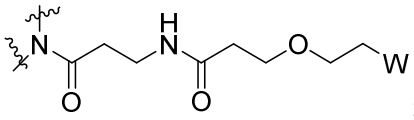
IL-2 conjugates in which the [AzK_L1_PEG30kD] is an L-amino acid having the structure of Formula (XVII).

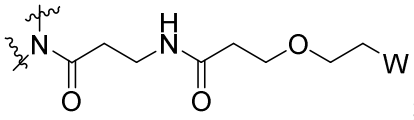
12. An IL-2 conjugate comprising the amino acid sequence of SEQ ID NO: 3, wherein at least one amino acid at position K34, F41, F43, K42, E61, P64, R37, T40, E67, Y44, V68, or L71 is replaced by the structure of Formula (I):

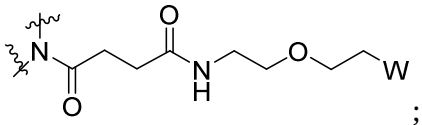


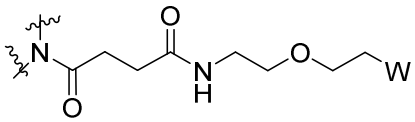
Formula (I)

wherein:

Z is CH_2 and Y is  ;

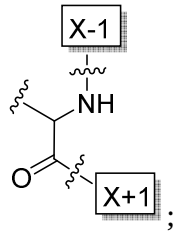
Y is CH_2 and Z is  ;

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.

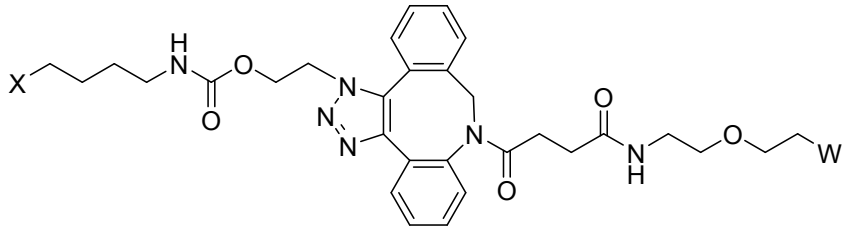
13. The IL-2 conjugate of claim 12, which is a pharmaceutically acceptable salt, solvate, or hydrate.

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

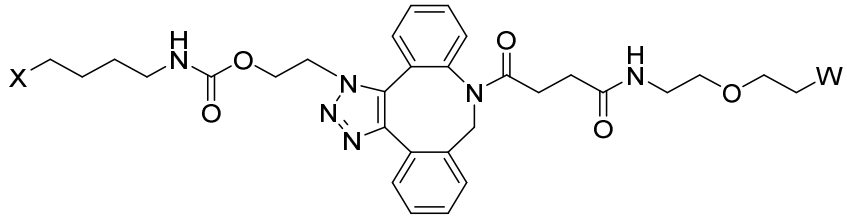
15. The IL-2 conjugate of claim 12, wherein the amino acid at position P64 is replaced by the structure of Formula (I).

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.

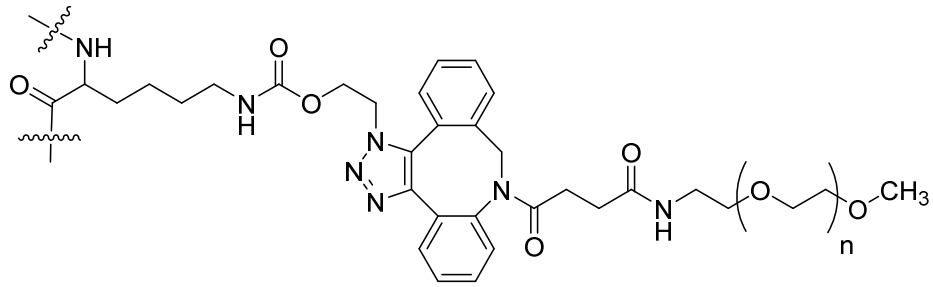
18. The IL-2 conjugate of claim 17, wherein W is a PEG group having a molecular weight of about 30 kDa.

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

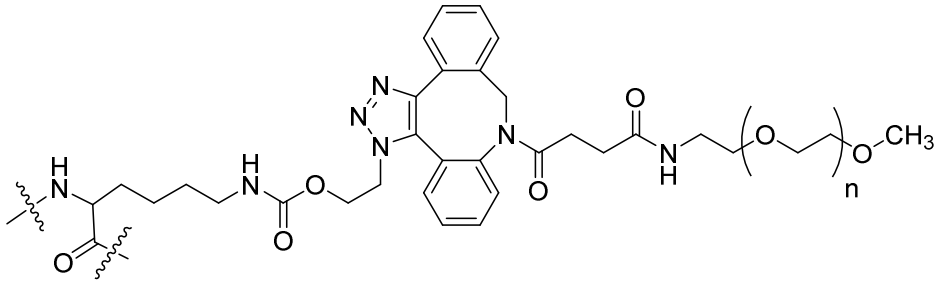
20. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 17 and a pharmaceutically acceptable excipient, wherein the mixture comprises IL-2 conjugates in which the structure of Formula (I) has the structure of Formula (IV) and IL-2 conjugates in which the structure of Formula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

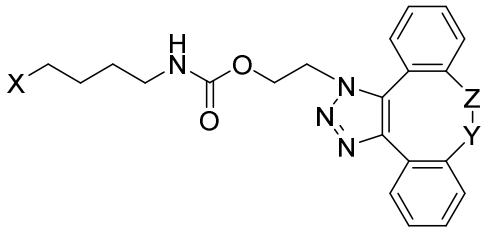
the wavy lines indicate covalent bonds to amino acid residues within SEQ ID NO: 3 that are not replaced.

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.

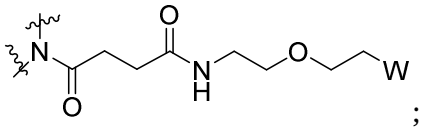
25. A pharmaceutical composition comprising a mixture of IL-2 conjugates of claim 22 and a pharmaceutically acceptable excipient, wherein the mixture comprises IL-2 conjugates in which amino acid P64 is replaced by the structure of Formula (XII) and IL-2 conjugates in which amino acid P64 is replaced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

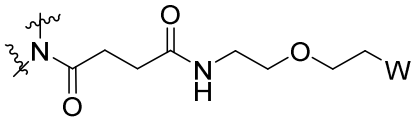


Formula (I)

wherein:

Z is CH_2 and Y is 

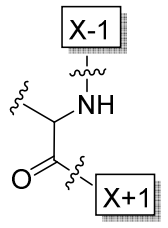
; or

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.