Claims

1. An IL-2 protein of formula (I)

   $(Tag^1)_y$ – $(Ala)_x$ – SEQ A – SEQ B – SEQ C – $(Tag^2)_z$ (I),

   wherein

   SEQ A has at least 89% sequence identity with SEQ ID NO:1;

   SEQ B has at least 76% sequence identity to SEQ ID NO:2 and comprises at least one glycosylation motif;

   SEQ C has at least 91% sequence identity to SEQ ID NO:4;

   Tag^1 and Tag^2 are independently a tag moiety;

   Ala is an alanine residue;

   x is 0 or 1;

   y is 0 or 1; and

   z is 0 or 1.

2. The IL-2 protein of claim 1, wherein SEQ A is selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:36.

3. The IL-2 protein of claim 1 or 2, wherein the at least one glycosylation motif in SEQ B is an N-glycosylation motif or an O-glycosylation motif.

4. The IL-2 protein of any one of claims 1 to 3, wherein the at least one N-glycosylation motif in SEQ B has the amino acid sequence $X_1X_2NX_3X_4$ (SEQ ID NO:254), wherein

   X_1 is any proteinogenic or non-proteinogenic amino acid or is absent;

   X_2 is any proteinogenic or non-proteinogenic amino acid or is absent;

   N is asparagine;

   X_3 is any proteinogenic or non-proteinogenic amino acid except proline; and

   X_4 is selected from the group consisting of threonine, serine and cysteine.

5. The IL-2 protein of any one of claims 1 to 4, wherein the at least one N-glycosylation motif in SEQ B is FGNST (SEQ ID NO:307) or FANST (SEQ ID NO:331).

6. The IL-2 protein of any one of claims 1 to 5, wherein SEQ B is selected from the group consisting of SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:214 and SEQ ID NO:215.

7. The IL-2 protein of any one of claims 1 to 6, wherein SEQ B has the sequence of SEQ ID NO:11.

8. The IL-2 protein of any one of claims 1 to 7, wherein SEQ C is selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:212.

9. The IL-2 protein of any one of claims 1 to 8, wherein x of formula (I) is 1.

10. The IL-2 protein of any one of claims 1 to 9, wherein y and z of formula (I) are 0.

11. The IL-2 protein of any one of claims 1 to 10, wherein the IL-2 protein has a sequence selected from the group consisting of SEQ ID NO:10; SEQ ID NO:13; SEQ ID NO:16; SEQ ID NO:19; SEQ ID NO:22; SEQ ID NO:25; SEQ ID NO:31; SEQ ID NO:34; SEQ ID NO:217; and SEQ ID NO:225.

12. The IL-2 protein of any one of claims 1 to 11, wherein the IL-2 protein has the sequence SEQ ID NO:10.

13. The IL-2 protein of any one of claims 1 to 12, wherein the IL-2 protein is a biased IL-2.

14. A conjugate comprising one or more of the IL-2 proteins of any one of claims 1 to 13.

15. The conjugate of claim 14, wherein the conjugate is an IL-2 conjugate or a pharmaceutically acceptable salt thereof of formula (Ia) or (Ib)

$$Z-\left(L^2-L^1-D\right)_x \quad \text{(Ia)},$$

$$D-\left(L^1-L^2-Z\right)_y \quad \text{(Ib)},$$

wherein

-D comprises the IL-2 protein of formula (I);

$-L^1-$ is a linker moiety covalently and reversibly attached to -D;

$-L^2-$ is a chemical bond or is a spacer moiety;

-Z is a polymeric moiety or a substituted fatty acid moiety;

x is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16; and

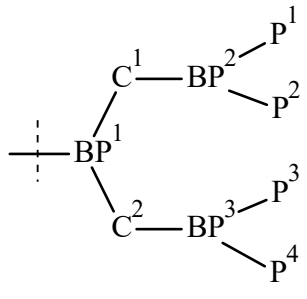
y is an integer selected from the group consisting of 2, 3, 4 and 5.

16. The conjugate or a pharmaceutically acceptable salt thereof of claim 14 or 15, wherein -Z is a polymeric moiety.

17. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 16, wherein -Z is a polymeric moiety having a molecular weight ranging from 1 kDa to 1000 kDa.

18. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 17, wherein -Z is a PEG-based polymeric moiety.

19. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 18, wherein -Z comprises a moiety of formula (A)



(A),

wherein

$-BP^1<$, $-BP^2<$, $-BP^3<$ are independently of each other selected from the group consisting of -N< and $-C(R^8)<$;

R^8 is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl;

$-P^1$, $-P^2$, $-P^3$, $-P^4$ are independently of each other a PEG-based chain comprising at least 40% PEG and having a molecular weight ranging from 3 to 40 kDa;

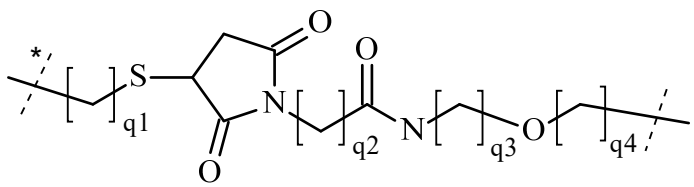
$-C^1-$, $-C^2-$ are independently of each other selected from the group consisting of C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more R^9, which are the same or different and wherein C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, $-C(O)N(R^{10})-$, $-S(O)_2N(R^{10})-$, $-S(O)N(R^{10})-$, $-S(O)_2-$, -S(O)-, $-N(R^{10})S(O)_2N(R^{10a})-$, -S-, $-N(R^{10})-$, $-OC(OR^{10})(R^{10a})-$, $-N(R^{10})C(O)N(R^{10a})-$, and $-OC(O)N(R^{10})-$;

each T is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, 8-to 30-membered carbopolycyclyl, and 8- to 30-membered heteropolycyclyl; wherein each T is independently optionally substituted with one or more R^9, which are the same or different;

each R^9 is independently selected from the group consisting of halogen, -CN, oxo (=O), $-COOR^{11}$, $-OR^{11}$, $-C(O)R^{11}$, $-C(O)N(R^{11}R^{11a})$, $-S(O)_2N(R^{11}R^{11a})$, $-S(O)N(R^{11}R^{11a})$, $-S(O)_2R^{11}$, $-S(O)R^{11}$, $-N(R^{11})S(O)_2N(R^{11a}R^{11b})$, $-SR^{11}$, $-N(R^{11}R^{11a})$, $-NO_2$, $-OC(O)R^{11}$, $-N(R^{11})C(O)R^{11a}$, $-N(R^{11})S(O)_2R^{11a}$, $-N(R^{11})S(O)R^{11a}$, $-N(R^{11})C(O)OR^{11a}$, $-N(R^{11})C(O)N(R^{11a}R^{11b})$, $-OC(O)N(R^{11}R^{11a})$, and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different; and

each R^{10}, R^{10a}, R^{11}, R^{11a} and R^{11b} is independently selected from the group consisting of -H, and C_{1-6} alkyl, wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

20. The conjugate or a pharmaceutically acceptable salt thereof of claim 19, wherein C^1 and C^2 are of formula (A-a)



(A-a),

wherein

the dashed line marked with the asterisk indicates attachment to BP^1;

the unmarked dashed line indicates attachment to BP^2 or BP^3, respectively;

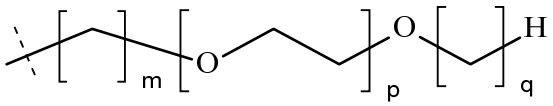
q1 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8;

q2 is selected from the group consisting of 1, 2, 3, 4, and 5;

q3 is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8; and

q4 is selected from the group consisting of 1, 2 and 3.

21. The conjugate or a pharmaceutically acceptable salt thereof of claim 19 or 20, wherein P^1, P^2, P^3 and P^4 are independently of each other of formula (A-b)



(A-b),

wherein

the dashed line indicates attachment to the remainder of -Z;

m is 0 or 1;

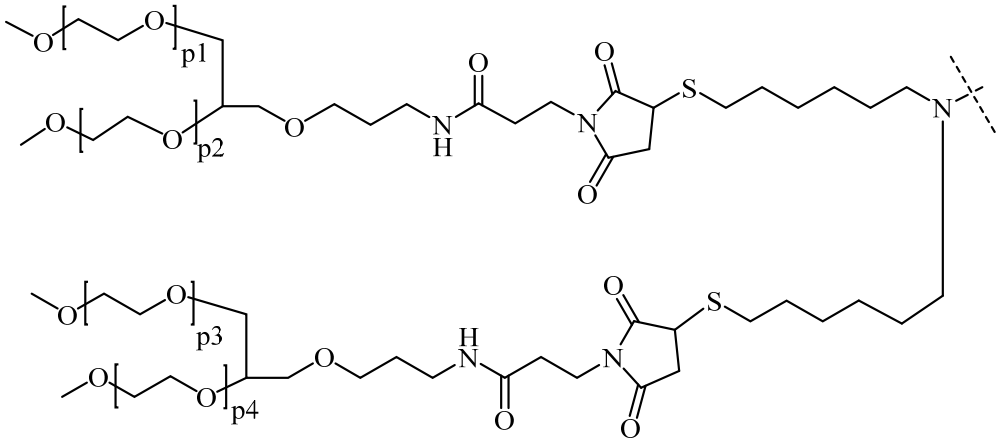
p is an integer ranging from 70 to 900; and

q is selected from the group consisting of 1, 2, 3, 4, 5, and 6.

22. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 19 to 21, wherein BP^1 is –N<.

23. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 19 to 22, wherein BP^2 and BP^2 are both –CH<.

24. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 23, wherein -Z comprises a moiety of formula (A-c):

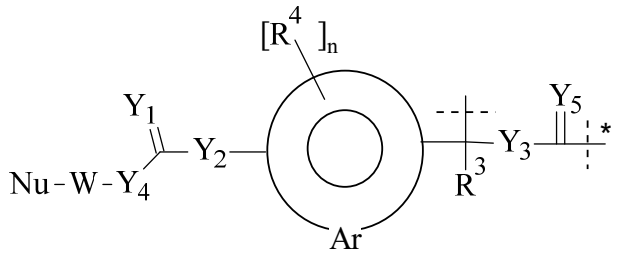


(A-c),

wherein

p1, p2, p3, p4 are independently of each other an integer ranging from 70 to 900.

25. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 24, wherein $-L^1-$ is of formula (IX-a):



(IX-a),

wherein

the dashed line marked with the asterisk indicates attachment to a nitrogen of -D and the unmarked dashed line indicates attachment to $-L^2-Z$;

n is 0, 1, 2, 3, or 4;

$=Y_1$, is selected from the group consisting of =O and =S;

$-Y_2-$ is selected from the group consisting of -O- and -S-;

$-Y_3-$ is selected from the group consisting of -O- and -S-;

$-Y_4-$ is selected from the group consisting of -O-, $-NR^5-$ and $-C(R^6R^{6a})-$;

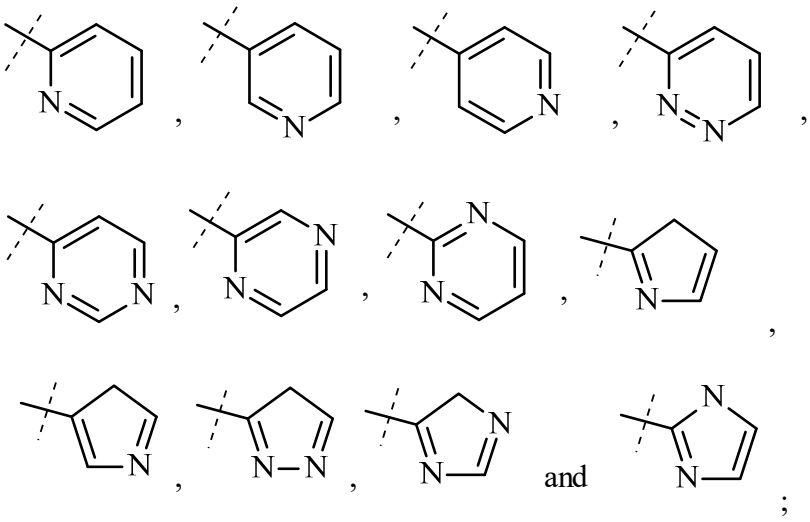
$=Y_5$ is selected from the group consisting of =O and =S;

$-R^3$, $-R^5$, $-R^6$, $-R^{6a}$ are independently of each other selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl;

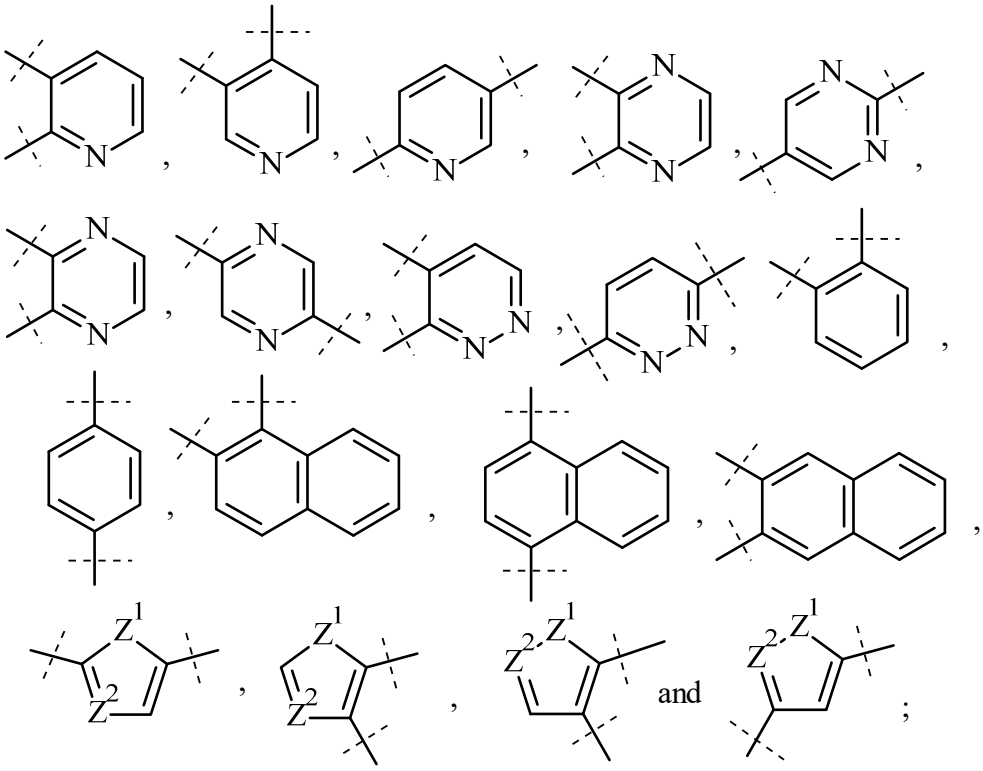
$-R^4$ is selected from the group consisting of methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl;

-W- is selected from the group consisting of C_{1-20} alkyl optionally interrupted by one or more groups selected from the group consisting of C_{3-10} cycloalkyl, 8- to 30-membered carbopolycyclyl, 3- to 10-membered heterocyclyl, -C(O)-, $-C(O)N(R^7)-$, -O-, -S- and $-N(R^7)-$;

-Nu is a nucleophile selected from the group consisting of $-N(R^7R^{7a})$, $-N(R^7OH)$, $-N(R^7)-N(R^{7a}R^{7b})$, $-S(R^7)$, -COOH,



-Ar- is selected from the group consisting of



wherein

dashed lines indicate attachment to the remainder of $-L^1-$,

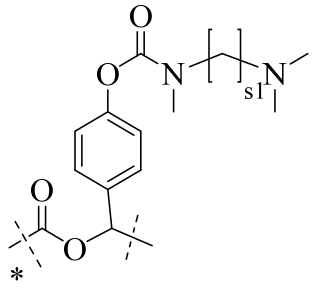
$-Z^1-$ is selected from the group consisting of -O-, -S- and $-N(R^7)-$, and

$-Z^2-$ is $-N(R^7)-$; and

$-R^7$, $-R^{7a}$, $-R^{7b}$ are independently of each other selected from the group consisting of -H, C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl;

wherein $-L^1-$ is optionally further substituted.

26. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 25, wherein $-L^1-$ is of formula (IX-c)



(IX-c),

wherein

the dashed line marked with the asterisk indicates attachment to a nitrogen of -D;

the unmarked dashed line indicates attachment to $-L^2-Z$; and

s1 is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

27. The conjugate or a pharmaceutically acceptable salt thereof of claim 26, wherein s1 is 3.

28. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 27, wherein $-L^2-$ is a spacer.

29. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 28, wherein $-L^2-$ is selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, $-C(O)N(R^{y1})-$, $-S(O)_2N(R^{y1})-$, $-S(O)N(R^{y1})-$, $-S(O)_2-$, -S(O)-, $-N(R^{y1})S(O)_2N(R^{y1a})-$, -S-, $-N(R^{y1})-$, $-OC(OR^{y1})(R^{y1a})-$, $-N(R^{y1})C(O)N(R^{y1a})-$, $-OC(O)N(R^{y1})-$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein -T-, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more $-R^{y2}$, which are the same or different and wherein C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, $-C(O)N(R^{y3})-$, $-S(O)_2N(R^{y3})-$, $-S(O)N(R^{y3})-$, $-S(O)_2-$, -S(O)-, $-N(R^{y3})S(O)_2N(R^{y3a})-$, -S-, $-N(R^{y3})-$, $-OC(OR^{y3})(R^{y3a})-$, $-N(R^{y3})C(O)N(R^{y3a})-$, and $-OC(O)N(R^{y3})-$;

$-R^{y1}$ and $-R^{y1a}$ are independently of each other selected from the group consisting of -H, -T, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein -T, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more $-R^{y2}$, which are

the same or different, and wherein C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, -C(O)N(R^{y4})-, -S(O)$_2$N(R^{y4})-, -S(O)N(R^{y4})-, -S(O)$_2$-, -S(O)-, -N(R^{y4})S(O)$_2$N(R^{y4a})-, -S-, -N(R^{y4})-, -OC(OR^{y4})(R^{y4a})-, -N(R^{y4})C(O)N(R^{y4a})-, and -OC(O)N(R^{y4})-;

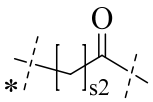
each T is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, 8-to 30-membered carbopolycyclyl, and 8- to 30-membered heteropolycyclyl; wherein each T is independently optionally substituted with one or more -R^{y2}, which are the same or different;

each -R^{y2} is independently selected from the group consisting of halogen, -CN, oxo (=O), -COOR^{y5}, -OR^{y5}, -C(O)R^{y5}, -C(O)N($R^{y5}R^{y5a}$), -S(O)$_2$N($R^{y5}R^{y5a}$), -S(O)N($R^{y5}R^{y5a}$), -S(O)$_2R^{y5}$, -S(O)R^{y5}, -N(R^{y5})S(O)$_2$N($R^{y5a}R^{y5b}$), -SR^{y5}, -N($R^{y5}R^{y5a}$), -NO$_2$, -OC(O)R^{y5}, -N(R^{y5})C(O)R^{y5a}, -N(R^{y5})S(O)$_2R^{y5a}$, -N(R^{y5})S(O)R^{y5a}, -N(R^{y5})C(O)OR^{y5a}, -N(R^{y5})C(O)N($R^{y5a}R^{y5b}$), -OC(O)N($R^{y5}R^{y5a}$), and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different; and

each -R^{y3}, -R^{y3a}, -R^{y4}, -R^{y4a}, -R^{y5}, -R^{y5a} and -R^{y5b} is independently selected from the group consisting of -H, and C_{1-6} alkyl, wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

30. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 29, wherein -L^2- is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O-, -T- and -C(O)N(R^{y1})-; and which C_{1-20} alkyl chain is optionally substituted with one or more groups independently selected from -OH, -T and -C(O)N($R^{y6}R^{y6a}$); wherein -R^{y1}, -R^{y6}, -R^{y6a} are independently selected from the group consisting of H and C_{1-4} alkyl and wherein T is selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, 8-to 30-membered carbopolycyclyl, and 8- to 30-membered heteropolycyclyl.

31. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 30, wherein -L^2- is of formula (IX-e)


(IX-e),

wherein

the dashed line marked with the asterisk indicates attachment to $-L^1-$;

the unmarked dashed line indicates attachment to -Z; and

s2 is an integer selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

32. The conjugate or a pharmaceutically acceptable salt thereof of claim 31, wherein s2 is 3.

33. A pharmaceutical composition comprising at least one IL-2 protein of any one of claims 1 to 13 or at least one conjugate or the pharmaceutically acceptable salt thereof of any one of claims 14 to 32 and at least one excipient.

34. The IL-2 protein of any one of claims 1 to 13, the conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 32, or the pharmaceutical composition of claim 33, for use as a medicament.

35. Use of the IL-2 protein of any one of claims 1 to 13, the conjugate or a pharmaceutically acceptable salt thereof of any one of claims 14 to 32, or the pharmaceutical composition of claim 33, in the manufacture of a medicament for treating cancer.

36. The use of claim 35, wherein the cancer is selected from the group consisting of sarcoma, chordoma, colon cancer, rectal cancer, colorectal cancer, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell cancer, basal cell cancer, adenocarcinoma, sweat gland cancer, sebaceous gland cancer, papillary cancer, papillary adenocarcinomas, cystadenocarcinoma, medullary cancer, bronchogenic cancer, renal cell cancer, hepatoma, bile duct cancer, choriocarcinoma, seminoma, embryonal cancer, Wilms' tumor, cervical cancer, testicular cancer, gastric cancer, non-small cell lung cancer, small cell lung cancer, bladder cancer, renal cell carcinoma, urothelial cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma,

oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, acute myeloid leukemia and leukemias.

37. The use of claim 35, wherein the medicament is to be administered to a patient prior to, simultaneously with, or after administration of one or more additional drug.

38. The use of claim 37, wherein the one or more additional drug is selected from the group consisting of pattern recognition receptor agonists (PRRA), cytotoxic/chemotherapeutic agents, immune checkpoint inhibitors or antagonists, immune checkpoint agonists, immune activating receptor agonists, multi-specific drugs, antibody-drug conjugates (ADC), antibody-adjuvant conjugates (AAC), radionuclides or targeted radionuclide therapeutics, DNA damage repair inhibitors, tumor metabolism inhibitors, pattern recognition receptor agonists, protein kinase inhibitors, chemokine and chemoattractant receptor agonists, chemokine or chemokine receptor antagonists, cytokine receptor agonists, death receptor agonists, CD47 or SIRPα antagonists, oncolytic drugs, signal converter proteins, epigenetic modifiers, tumor peptides or tumor vaccines, heat shock protein (HSP) inhibitors, proteolytic enzymes, ubiquitin and proteasome inhibitors, adhesion molecule antagonists, hormones including hormone peptides and synthetic hormones, and adoptive cellular therapies such as Tumor Infiltrating Lymphocyte (TIL) therapy, Chimeric Antigen Receptor (CAR) therapy, T cell therapy, Natural Killer (NK) cell therapy, CAR-T therapy, CAR-NK therapy, CAR-γδ therapy, CAR-Macrophage therapy, or any other cellular therapy with a genetically modified or genetically unmodified immune cell type.

39. An oligonucleotide encoding the IL-2 protein of any one of claims 1 to 13.

40. The oligonucleotide of claim 39, wherein the oligonucleotide is selected from the group consisting of DNA, RNA and cDNA.

41. Use of the oligonucleotide of claim 39 or 40 in the manufacture of a medicament for the treatment of a disease that can be treated with IL-2, in particular with a biased IL-2.

42. The use of claim 41, wherein the medicament is to be delivered into a host cell in vivo or ex vivo.

43. The use of claim 42, wherein the host cell is an immune cell.

44. The oligonucleotide of claim 39 or 40 for use in therapy.