

However, manufacturing proteins, such as IL-2, with an additional cysteine may be problematic, because such proteins may have a tendency to aggregate and it may be difficult or impossible to ensure proper renaturation of such protein with sufficient quality and in sufficient amounts.

It is therefore an object of the present invention to at least partially overcome the above-mentioned disadvantage.

In an example, this object is achieved with an IL-2 protein sequence of formula (I)

Ala – SEQ A – Cys* – SEQ B (I),

wherein

SEQ A has at least 94% sequence identity to SEQ ID NO:1;

SEQ B has at least 94% sequence identity to SEQ ID NO:2;

Ala is an alanine residue; and

Cys* is a cysteine residue.

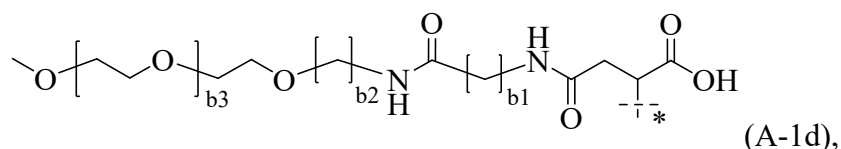
It was surprisingly found that the addition of an N-terminal alanine residue significantly improved yields when such IL-2 protein is expressed as a soluble protein in, for example, a mammalian expression system or a yeast expression system, compared to the corresponding IL-2 sequence without such N-terminal alanine. The cysteine marked with the asterisk may be a free cysteine, i.e. one where the thiol is not part of a disulfide bond, or it may be coupled to a thiol-comprising compound, such as to a cysteine, via a disulfide bridge.

Such IL-2 of formula (I) may be a biased IL-2.

In one aspect, the present disclosure provides a conjugate D-L¹-L²-Z or a pharmaceutically acceptable salt thereof

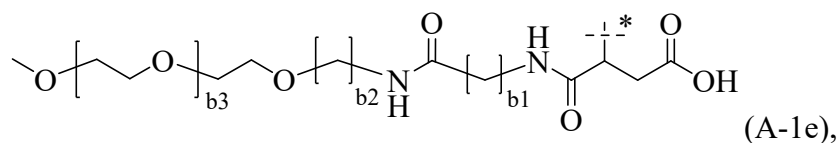
wherein

-D comprises an IL-2 moiety of SEQ ID NO:14, wherein the IL-2 moiety comprises a moiety M_{mod} of formula (A-1d)



3a

or (A-1e)



wherein

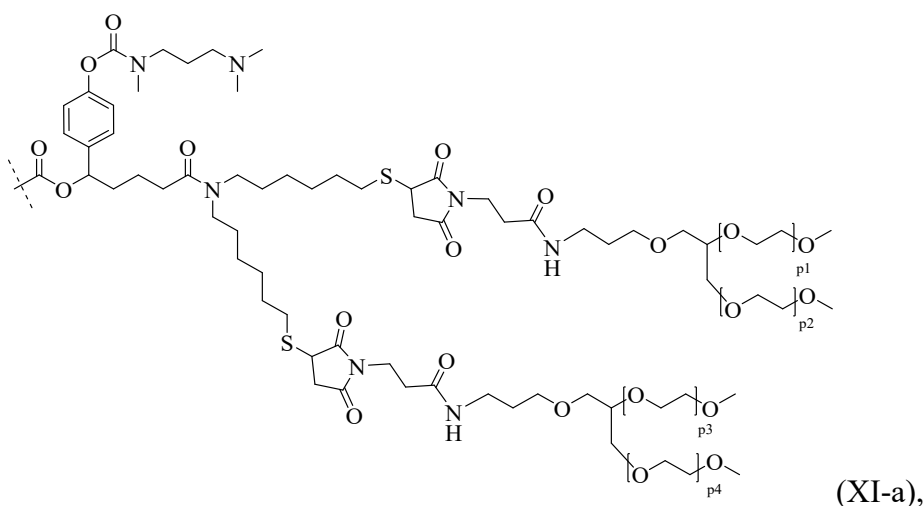
each b1 is 2;

each b2 is 2 or 3; and

each b3 is an integer ranging from 100 to 125

wherein the dashed line with the asterisk indicates attachment to a sulfur atom of a cysteine at position 38; and

-L¹-L²-Z is a moiety of formula (XI-a)



wherein

the dashed line indicates attachment to a nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety; and

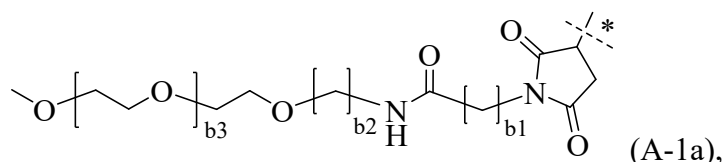
p1, p2, p3 and p4 are independently of each other an integer ranging from 220 to 240.

In another aspect, the present disclosure provides a conjugate D-L¹-L²-Z or a pharmaceutically acceptable salt thereof

wherein

-D comprises an IL-2 moiety of SEQ ID NO:14, wherein the IL-2 moiety comprises a moiety M_{mod} of formula (A-1a)

3b



wherein

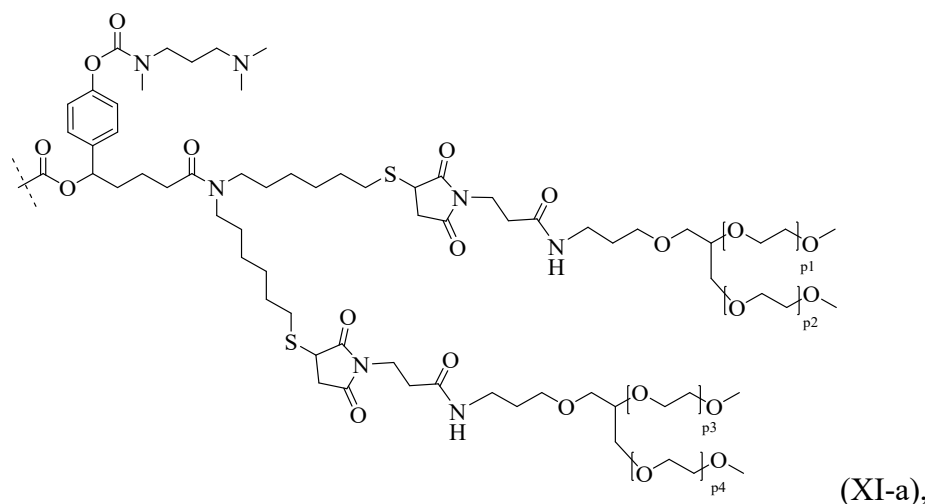
each b1 is 2;

each b2 is 2 or 3; and

5 each b3 is an integer ranging from 100 to 125

wherein the dashed line with the asterisk indicates attachment to a sulfur atom of a cysteine at position 38; and

-L¹-L²-Z is a moiety of formula (XI-a)



10 wherein

the dashed line indicates attachment to a nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety; and

15 p1, p2, p3 and p4 are independently of each other an integer ranging from 220 to 240.

In yet another aspect, the present disclosure provides a plurality of the conjugates or pharmaceutically acceptable salts thereof as defined herein, wherein conjugates are present as a mixture and wherein the mixture comprises

- 20 (i) at least one conjugate comprising an IL-2 moiety of SEQ ID NO:14, to which a moiety M_{mod} of formula (A-1d) is conjugated to the sulfur atom of the cysteine at position 38, wherein b1 is 2, b2 is 2 or 3 and b3 is an integer ranging from about 100 to 125, and to which IL-2 moiety a moiety of formula (XI-a)

is conjugated to the nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety and p1, p2, p3 and p4 range from 220 to 240; and

- (ii) at least one conjugated comprising an IL-2 moiety of SEQ ID NO:14, to which a moiety M_{mod} of formula (A-1e) is conjugated to the sulfur atom of the cysteine at position 38, wherein b1 is 2, b2 is 2 or 3 and b3 is an integer ranging from about 100 to 125, and to which IL-2 moiety a moiety of formula (XI-a) is conjugated to the nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety and p1, p2, p3 and p4 range from 220 to 240.

In yet another aspect, the present disclosure provides a pharmaceutical composition comprising at least one conjugate or the pharmaceutically acceptable salt thereof as defined herein or the plurality of conjugates or pharmaceutically acceptable salts thereof as defined herein, and at least one excipient.

In yet another aspect, the present disclosure provides the conjugate or a pharmaceutically acceptable salt thereof as defined herein, the plurality of conjugates or pharmaceutically acceptable salts thereof as defined herein or the pharmaceutical composition as defined herein for use in therapy.

In yet another aspect, the present disclosure provides use of the conjugate or a pharmaceutically acceptable salt thereof as defined herein, the plurality of conjugates or pharmaceutically acceptable salts thereof as defined herein or the pharmaceutical composition as defined herein in the manufacture of a medicament for the treatment of cancer.

Within the present invention the terms are used having the meaning as follows.

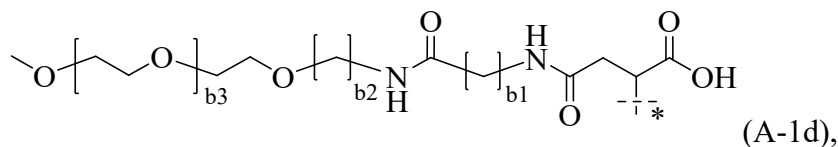
In general, the term “interleukin-2” or “IL-2” refers to all IL-2 proteins, preferably from mammalian species, more preferably from primate species and most preferably from human, as well as their variants, analogs, orthologs, homologs, and derivatives and fragments thereof, that are characterized by playing a central role in lymphocyte generation, survival and homeostasis, and also encompasses naturally occurring variants of IL-2, e.g. splice variants or allelic variants.

Claims

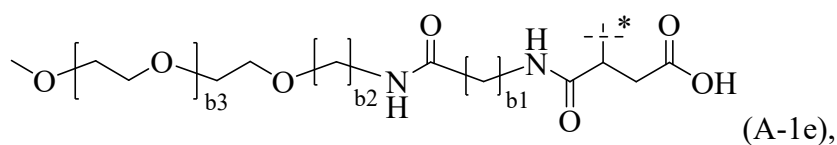
1. A conjugate D-L¹-L²-Z or a pharmaceutically acceptable salt thereof

wherein

-D comprises an IL-2 moiety of SEQ ID NO:14, wherein the IL-2 moiety comprises a moiety M_{mod} of formula (A-1d)



or (A-1e)



wherein

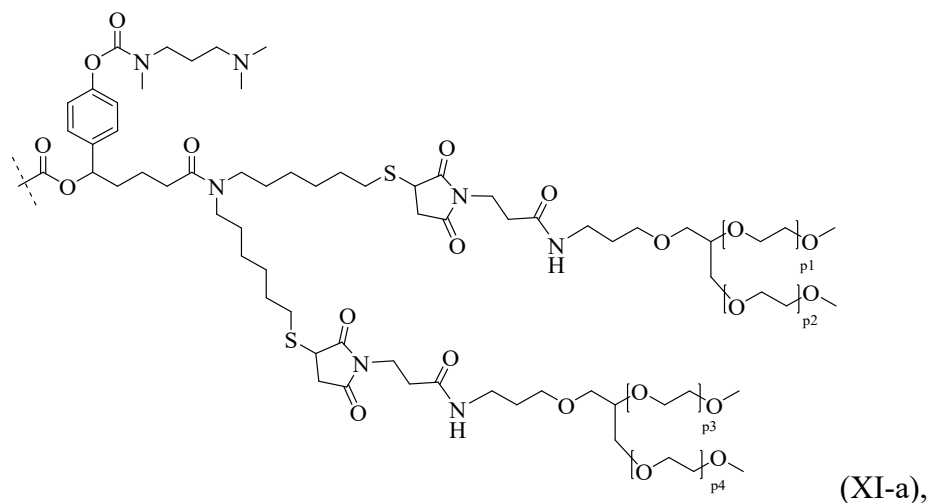
each b1 is 2;

each b2 is 2 or 3; and

each b3 is an integer ranging from 100 to 125

wherein the dashed line with the asterisk indicates attachment to a sulfur atom of a cysteine at position 38; and

-L¹-L²-Z is a moiety of formula (XI-a)



wherein

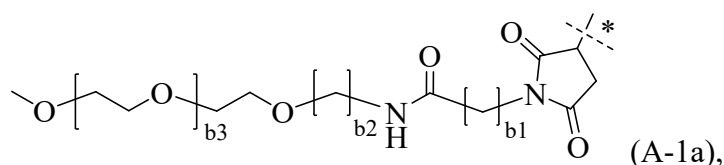
the dashed line indicates attachment to a nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety; and

p1, p2, p3 and p4 are independently of each other an integer ranging from 220 to 240.

2. A conjugate D-L¹-L²-Z or a pharmaceutically acceptable salt thereof

wherein

- 5 -D comprises an IL-2 moiety of SEQ ID NO:14, wherein the IL-2 moiety comprises a moiety M_{mod} of formula (A-1a)



wherein

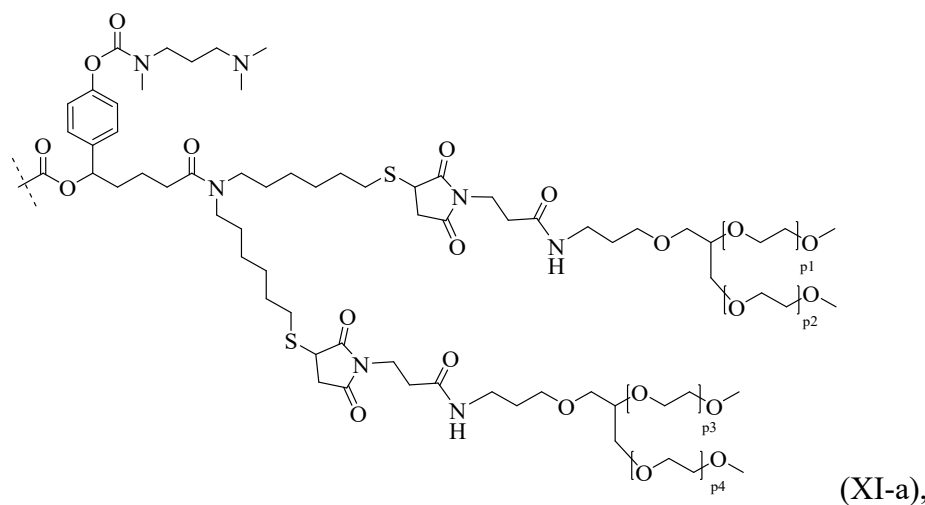
each b1 is 2;

- 10 each b2 is 2 or 3; and

each b3 is an integer ranging from 100 to 125

wherein the dashed line with the asterisk indicates attachment to a sulfur atom of a cysteine at position 38; and

-L¹-L²-Z is a moiety of formula (XI-a)



wherein

the dashed line indicates attachment to a nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety; and

- 20 p1, p2, p3 and p4 are independently of each other an integer ranging from 220 to 240.

3. The conjugate or a pharmaceutically acceptable salt thereof of claims 1 or 2, wherein attachment of M_{mod} to the IL-2 moiety is via a stable linkage.

4. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 3, wherein the IL-2 moiety of SEQ ID NO:14 is O-glycosylated and/or N-glycosylated.

5. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 4, wherein the IL-2 moiety of SEQ ID NO:14 is O-glycosylated.

6. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 5, wherein the IL-2 moiety of SEQ ID NO:14 is O-glycosylated at the N-terminal end, corresponding to positions 1 to 8.

7. The conjugate or a pharmaceutically acceptable salt thereof of claim 6, wherein the IL-2 moiety of SEQ ID NO:14 is glycosylated with a glycoform, which is (Hex)₁(HexNAc)₁(NeuAc)₂ or (Hex)₁(HexNAc)₁(NeuAc)₁.

8. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 7, wherein the IL-2 moiety of SEQ ID NO:14 is obtained from CHO cells.

9. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 8, wherein the dashed line indicates attachment to a nitrogen atom of an amine of a lysine side chain of -D.

10. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 9, wherein b3 is about 112.

11. A plurality of the conjugates or pharmaceutically acceptable salts thereof of any one of claims 1 to 10, wherein conjugates are present as a mixture and wherein the mixture comprises

- (i) at least one conjugate comprising an IL-2 moiety of SEQ ID NO:14, to which a moiety M_{mod} of formula (A-1d) is conjugated to the sulfur atom of the cysteine at position 38, wherein b1 is 2, b2 is 2 or 3 and b3 is an integer ranging from about 100 to 125, and to which IL-2 moiety a moiety of formula (XI-a) is conjugated to the nitrogen atom of a primary amine of the N-terminus or a

lysine side chain residue of the IL-2 moiety and p1, p2, p3 and p4 range from 220 to 240; and

- (ii) at least one conjugated comprising an IL-2 moiety of SEQ ID NO:14, to which a moiety M_{mod} of formula (A-1e) is conjugated to the sulfur atom of the cysteine at position 38, wherein b1 is 2, b2 is 2 or 3 and b3 is an integer ranging from about 100 to 125, and to which IL-2 moiety a moiety of formula (XI-a) is conjugated to the nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety and p1, p2, p3 and p4 range from 220 to 240.

12. The plurality of conjugates or pharmaceutically acceptable salts thereof of claim 11, wherein the mixture further comprises at least one conjugate comprising an IL-2 moiety of SEQ ID NO:14, to which a moiety M_{mod} of formula (A-1a) is conjugated to the sulfur atom of the cysteine at position 38, wherein b1 is 2, b2 is 2 or 3 and b3 is an integer ranging from about 100 to 125, and to which IL-2 moiety a moiety of formula (XI-a) is conjugated to the nitrogen atom of a primary amine of the N-terminus or a lysine side chain residue of the IL-2 moiety and p1, p2, p3 and p4 range from 220 to 240.

13. The plurality of conjugates or their pharmaceutically acceptable salts thereof of claims 11 or 12, wherein p1, p2, p3 and p4 in each conjugate of the mixture is about 112.

14. A pharmaceutical composition comprising at least one conjugate or the pharmaceutically acceptable salt thereof of any one of claims 1 to 10 or the plurality of conjugates or pharmaceutically acceptable salts thereof of any one of claims 11 to 13, and at least one excipient.

15. The conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 10, the plurality of conjugates or pharmaceutically acceptable salts thereof of any one of claims 11 to 13 or the pharmaceutical composition of claim 14 for use in therapy.

16. Use of the conjugate or a pharmaceutically acceptable salt thereof of any one of claims 1 to 10, the plurality of conjugates or pharmaceutically acceptable salts thereof of any one of claims 11 to 13 or the pharmaceutical composition of claim 14 in the manufacture of a medicament for the treatment of cancer.

17. The use of claim 16, 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.
18. The use of claims 16 or 17, wherein the conjugate or a pharmaceutically acceptable salt thereof or the pharmaceutical composition is to be administered to the patient prior to, simultaneously with, or after administration of one or more additional drug.
19. The use of claim 18, 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 SIRP α 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- $\gamma\delta$ therapy, CAR-Macrophage

therapy, or any other cellular therapy with a genetically modified or genetically unmodified immune cell type.

- 5 20. The use of any one of claim 16 to 19, wherein the conjugate or a pharmaceutically acceptable salt thereof or the pharmaceutical composition results in an expansion of $\gamma\delta$ T cells of at least 100-fold over baseline.