

INTERLEUKIN-2 VARIANT PROTEINS THAT FACILITATE COVALENT CHEMICAL CONJUGATION AND USES THEREOF

TECHNICAL FIELD

- 5 [0001] The disclosure relates to mutational variants of the IL-2 protein that facilitate chemical conjugation and their potential use as treatments for cancer or inflammatory disease.

BACKGROUND

- 10 [0002] In immune therapy, achieving a viable therapeutic index with systemically-dosed, native, unmodified cytokines is notoriously difficult. This is due to the inherently high potency of cytokine molecules, coupled with the large population of responsive immune cells that are found in non-diseased tissues, some of which may respond differently when bound by the same signaling molecules, at any given concentration.
- 15 Furthermore, the induction of strong cytokine signaling in non-diseased tissue often causes unwanted side effects.

- [0003] Interleukin-2 (IL-2) is an example of a cytokine protein that regulates the activities of white blood cells (e.g. T cells and NK cells) that are responsible for immunity, responses to microbial infections, and in discriminating between foreign
- 20 ("non-self") and "self". IL-2 is therefore a molecule of strong potential therapeutic interest in both pro-inflammatory (e.g. in cancer) and anti-inflammatory (auto-immune disease) settings. IL-2 mediates its effects by binding to the IL-2 receptor complex, thereby stimulating the growth of immune cells such as helper, cytotoxic and regulatory T cells. The IL-2 receptor complex (IL-2R) consists of three chains, termed alpha (CD25 high
- 25 affinity, non-signaling), beta (CD122 moderate affinity, signaling) and gamma (CD132, low affinity, signaling). Heterodimerization of the β and γ subunits of IL-2R is essential for signaling in T cells. IL-2 can therefore signal either via intermediate-affinity dimeric

CD122/CD132 IL-2R ($K_d \sim 10^{-9}$ M) or high-affinity trimeric CD25/CD122/CD132 IL-2R ($K_d \sim 10^{-11}$ M) complexes. Differential expression levels of these two forms of IL-2R complex are found on NK cells, memory T cells, regulatory T cells and activated T cells, leading to pleiotrophic effects of IL-2, dependent on concentration of cytokine and dominance of different cell populations and concurrent alternative signals in any given tissue (diseased or normal). Overstimulation of regulatory T cells in cancer therapy with IL-2 may inhibit the desired stimulation of cytotoxic T effector cells. Equally, rapid overstimulation of effector T cells throughout the system may lead to dramatic toxicity. This intrinsic complexity of IL2 signaling on different cell classes has compounded the difficulty of using IL-2 as a soluble protein therapeutic.

[0004] For use as potential therapeutic agents, IL-2 proteins have commonly been synthesized as free cytokine, or antibody fusion proteins, but these molecules have suffered from high toxicity, poor biodistribution and/or poor therapeutic index. IL-2 and IL-2 antibody fusions could be made more tolerable and effective therapeutic agents *e.g.*, by improving their half-life in man, coupled with reduced binding affinity to one or more of its 3 known receptors. This would allow the effective stimulation of the desired effector cells of the immune system, while minimizing stimulation of undesired cells. For example, a molecule which has reduced or eliminated affinity for CD25, coupled with conditional affinity for CD122, may exhibit reduced ability to stimulate Treg cells (which are CD25-high) and more effectively stimulate Teff cells (CD25-low). This is a potentially beneficial phenotype in a cancer setting where one would seek to stimulate Teff cells over Treg, thereby stimulating anti-cancer therapeutic activity. In contrast, a molecule which has native (high) affinity for CD25, coupled with reduced affinity for CD122, may exhibit enhanced ability to stimulate Treg cells (which are CD25-high) and more effectively minimize stimulation of Teff cells (CD25-low). This is a potentially beneficial phenotype in an inflammatory disease setting where one would seek to stimulate Treg cells over Teff, thereby stimulating anti-inflammatory therapeutic activity.

[0005] However, the construction of IL-2 derived molecules with the correct combination of expression characteristics, purification quality, stability, solubility, and controlled receptor signaling is far from trivial. An ideal method would combine facile

expression of the protein in the highly established CHO cell platform, purification via standard manufacturing column processes and site-specific conjugation at an optimal site (or sites), with a moiety that modulates both receptor binding activity and pharmacokinetics. There is, therefore, a need for engineered forms of IL-2 proteins that
5 facilitate simplified and reproducible covalent modification, creating molecules with activity specifically optimized to achieve the desired pharmacology in the diseased tissue environment.

[0006] The Inventors have developed new mutant IL-2, derived from human IL-2, comprising non-endogenous transglutaminase motifs. Said transglutaminase motifs
10 enable to conjugate moieties in a simple and reproducible way, by transglutamination.

SUMMARY

[0007] The present invention relates to a mutant IL-2 derived from human IL-2 with the sequence SEQ ID NO: 1 or from a variant thereof, comprising at least one non-
15 endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif is inserted by:

- insertion of a Q residue at one or more of the following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, Q57, H79, E95, E100, E110 and S130, and/or
20 - insertion of a motif at the N-terminus or C-terminus, wherein said motif comprises a sequence selected from the group comprising or consisting of LQS and TQG,

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation), (ii) a substitution of the
25 glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S

mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0008] In one embodiment, said at least one non-endogenous transglutaminase motif is inserted by insertion of a Q residue at one or more of the following positions by reference
5 to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, Q57, H79, E95, E100, S130.

[0009] In one embodiment, said at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of X₁LQ wherein X₁ is A, L, H, I or V; NX₂Q wherein X₂ is Y, H or F; YRQ, LTQ; AQX₃ wherein X₃ is A or E, TEQ, LFQ; GSQ and VIQ, preferably comprising or consisting of
10 X₁LQ wherein X₁ is L, H, I or V; NX₂Q wherein X₂ is Y, H or F; YRQ, LTQR (SEQ ID NO: 2); LFQ; GSQ and VIQ.

[0010] In one embodiment, said at least one non-endogenous transglutaminase motif comprises or consists of:

- a sequence selected from the group comprising or consisting of
15 LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD (SEQ ID NO: 5), MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO: 7), NNYQNP (SEQ ID NO: 8), NNHQNP (SEQ ID NO: 9), LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), LLQC (SEQ ID NO: 13), KNFQLRPRD (SEQ ID NO: 14),
20 KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), VLQ, LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ, NNYRQP (SEQ ID NO: 102), NRYRQP (SEQ ID NO: 103), MLTQGF (SEQ ID NO: 104), LAQELK (SEQ ID NO: 105), LAQALK (SEQ ID NO: 106), KATEQK (SEQ ID NO: 107), EYALQ (SEQ ID NO: 108) and
25 DYALQ (SEQ ID NO: 109), preferably comprising or consisting of LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD (SEQ ID NO: 5), MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO: 7), NNYQNP (SEQ ID NO: 8), NNHQNP (SEQ ID NO: 9), LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), LLQC (SEQ ID NO: 13), KNFQLRPRD (SEQ ID NO: 14),
30

- KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), VLQ, LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ, NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103), and/or
- 5 - a sequence selected from the group comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), TQGAPTSSSTKKTQ (SEQ ID NO: 41), LQSPTSSTKKTQ (SEQ ID NO: 42), TQGASSSTKKTQ (SEQ ID NO: 43), TQGASSTKKTQ (SEQ ID NO: 44), and TQGASTKKTQ (SEQ ID NO: 45), preferably wherein said sequence is added at the N-terminus, and/or
 - 10 - a sequence selected from the group comprising or consisting of GGTQGA (SEQ ID NO: 46), GGLQSP (SEQ ID NO: 47), GLQSP (SEQ ID NO: 48), LQSP (SEQ ID NO: 49) and QGA, preferably wherein said sequence is added at the C-terminus.

[0011] In one embodiment, said at least one non-endogenous transglutaminase motif is inserted by:

- insertion of a Q residue at one or more of the following positions by reference to SEQ ID NO: 1: N33, T41, F42, H79, E100, and /or
- insertion of a motif at the N-terminus or C-terminus, wherein said motif comprises or consists of a sequence selected from the group comprising or consisting of LQS and TQG.

[0012] In one embodiment, said at least one non-endogenous transglutaminase motif comprises or consists of:

- 25 - a sequence selected from the group comprising or consisting of LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), GSQST (SEQ ID NO: 18), NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103), and/or
- 30 - a sequence selected from the group comprising or consisting of LQS and TQG, preferably wherein said sequence is added at the C-terminus or at the N-terminus.

[0013] In one embodiment, said at least one non-endogenous transglutaminase motif comprises or consists of:

- 5 - a sequence selected from the group comprising or consisting of LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), GSQST (SEQ ID NO: 18), NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103) , and/or
- 10 - a sequence selected from the group comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), LQSPTSSSTKKTQ (SEQ ID NO: 42), and TQGASSTKKTQ (SEQ ID NO: 44), preferably wherein said sequence is added at the N-terminus, and/or
- a sequence selected from the group comprising or consisting of GGLQSP (SEQ ID NO: 47), GLQSP (SEQ ID NO: 48), LQSP (SEQ ID NO: 49) and QGA, preferably wherein said sequence is added at the C-terminus.

15 [0014] In one embodiment, the mutant comprises or consists of a sequence selected from the group comprising or consisting of SEQ ID NO: 19 to SEQ ID NO: 39, SEQ ID NO: 51 to SEQ ID NO: 63, SEQ ID NO: 110 to SEQ ID NO: 117, more preferably comprising or consisting of SEQ ID NO: 19 to SEQ ID NO: 39, SEQ ID NO: 51 to SEQ ID NO: 63 and SEQ ID NOs: 110 and 111.

20 [0015] In one embodiment, the mutant comprises or consists of a sequence selected from the group comprising or consisting of SEQ ID NO: 30, SEQ ID NO: 20, SEQ ID NO: 19, SEQ ID NO: 21 to SEQ ID NO: 29, SEQ ID NO: 31 to SEQ ID NO: 39, SEQ ID NO: 51 to SEQ ID NO: 63, SEQ ID NO: 110 to SEQ ID NO: 117, more preferably comprising or consisting of SEQ ID NO: 19 to SEQ ID NO: 39, SEQ ID NO: 51 to SEQ ID NO: 63 and
25 SEQ ID NOs: 110 and 111.

[0016] In one embodiment, the mutant comprises or consists of a sequence selected from the group comprising or consisting of SEQ ID NO: 26 to SEQ ID NO: 28, SEQ ID NO: 31 to SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 58 to SEQ ID NO: 63 and SEQ ID NOs: 110 and 111.

[0017] In one embodiment, the mutant comprises or consists of a sequence selected from the group comprising or consisting of SEQ ID NO: 26 to SEQ ID NO: 28, SEQ ID NO: 30 to SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, and SEQ ID NO: 58 to SEQ ID NO: 63 and SEQ ID NOs: 110 and 111.

- 5 [0018] In one embodiment, the mutant IL-2 further comprises a substitution of the F42 residue by reference to SEQ ID NO: 1 into any other amino acid, more preferably a substitution of the F42 residue into an alanine residue.

[0019] The present invention also relates to a fusion protein comprising a mutant IL-2 as described hereinabove, and further comprising at least one masking moiety, wherein
10 the at least one masking moiety is linked to a Q residue of the at least one non-endogenous transglutaminase motif of the mutant IL-2 with a cleavable linker.

[0020] In one embodiment, the masking moiety is a polyethylene glycol (PEG), preferably a linear or a branched PEG.

[0021] In one embodiment, the fusion protein further comprises an antibody or antigen-
15 binding fragment thereof or an antibody mimetic, preferably wherein the antibody or antigen-binding fragment thereof or antibody mimetic is linked to the mutant IL-2 with a linker.

[0022] The present invention also relates to a fusion protein as described hereinabove, for use as a medicament.

20 [0023] The present invention also relates to a fusion protein as described hereinabove, for use in treating a cancer or an inflammatory disease.

DEFINITIONS

[0024] In the present invention, the following terms have the following meanings:

[0025] “**About**”, preceding a figure encompasses plus or minus 10%, or less, of the value of said figure. It is to be understood that the value to which the term “about” refers is itself also specifically, and preferably, disclosed.

[0026] “**Antibody**” and “**immunoglobulin**”, as used herein, may be used interchangeably and refer to a protein having a combination of two heavy and two light chains whether or not it possesses any relevant specific immunoreactivity. “Antibodies” refers to such assemblies which have significant known specific immunoreactive activity to an antigen of interest. Antibodies and immunoglobulins comprise light and heavy chains, with or without an interchain covalent linkage between them. Basic immunoglobulin structures in vertebrate systems are relatively well understood. The generic term “immunoglobulin” comprises five distinct classes of antibody that can be distinguished biochemically. Although the following discussion will generally be directed to the IgG class of immunoglobulin molecules, all five classes of antibodies are within the scope of the present invention. With regard to IgG, immunoglobulins comprise two identical light polypeptide chains of molecular weight of about 23 kDa, and two identical heavy chains of molecular weight of about 53-70 kDa. The four chains are joined by disulfide bonds in a “Y” configuration wherein the light chains bracket the heavy chains starting at the mouth of the “Y” and continuing through the variable region. The light chains of an antibody are classified as either kappa (κ) or lambda (λ). Each heavy chain class may be bonded with either a κ or λ light chain. In general, the light and heavy chains are covalently bonded to each other, and the “tail” regions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. Those skilled in the art will appreciate that heavy chains are classified as gamma (γ), mu (μ), alpha (α), delta (δ) or epsilon (ϵ) with some subclasses among them (*e.g.*, γ 1- γ 4). It is the nature of this chain that determines the “class” of the antibody as IgG, IgM, IgA IgD or IgE, respectively. The immunoglobulin subclasses or “isotypes” (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, etc.) are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily

discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the present invention. As indicated above, the variable region of an antibody allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the light chain variable domain (VL domain) and heavy chain variable domain (VH domain) of an antibody combine to form the variable region that defines a three-dimensional antigen binding site. This quaternary antibody structure forms the antigen binding site presents at the end of each arm of the “Y”. More specifically, the antigen binding site is defined by three complementarity determining regions (CDRs) on each of the VH and VL chains.

10 [0027] “**Antigen-binding fragment**”, as used herein, refers to a part or region of an antibody which comprises fewer amino acid residues than the whole antibody. An “antigen-binding fragment” binds antigen and/or competes with the whole antibody from which it derives for antigen binding (*e.g.*, specific binding to human CD25). Antigen-binding fragments encompasses, without any limitation, a single chain antibody, a dimeric single chain antibody, a Fv, a Fab, a Fab', a Fab'-SH, a F(ab)'₂, a Fd, a nanobody, 15 a domain antibody, and a unibody.

[0028] “**Derived from**” as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connote or include a process or source 20 limitation on a first molecule that is derived from a second molecule. For example, in the case of mutant IL-2 that is derived from human IL-2, the mutant retains sufficient IL-2 structure such that it has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connote or include a limitation to a particular process of producing the mutant IL-2.

25 [0029] “**Diabodies**”, as used herein, refer to small antibody fragments prepared by constructing scFv fragments with short linkers (about 5-10 residues) between the VH and VL such that inter-chain but not intra-chain pairing of the variable domains is achieved, resulting in a bivalent fragment, *i.e.*, fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two “crossover” scFv fragments in which the

VH and VL of the two antibodies are present on different polypeptide chains. Diabodies are described, for example, in patent EP0404097 or patent application WO1993011161.

[0030] “**Domain antibodies**” refer to the smallest functional binding units of antibodies, corresponding to the variable regions of either the heavy or light chains of antibodies.

- 5 [0031] “**Fab**” refers to a monovalent fragment containing the following regions: VH, VL, CH1 and CL, linked by an intramolecular disulfide bond.

[0032] As used herein, “**F(ab')₂**” refers to a fragment containing two antigen-binding regions joined by disulfides bonds.

- 10 [0033] As used herein, “**Fab**” refers to a fragment obtained by the reduction of F(ab')₂ fragments.

[0034] “**Fd fragment**” refers to the heavy chain of the Fab fragment, comprising the VH and CH1 regions.

- 15 [0035] “**Fv**”, as used herein, refers to the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one VH and one VL in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (three loops each from the heavy and light chain) that contribute to antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower
20 affinity than the entire binding site.

- [0036] “**Humanized antibody or antigen-binding fragment thereof**”, as used herein, refers to a chimeric antibody or antigen-binding fragment thereof which contains minimal sequence derived from a non-human immunoglobulin. It includes antibodies made by a non-human cell having variable and constant regions which have been altered to more
25 closely resemble antibodies that would be made by a human cell, *e.g.*, by altering the non-human antibody amino acid sequence to incorporate amino acids found in human germline immunoglobulin sequences. Humanized antibodies or antigen-binding fragments thereof comprised in a fusion protein according to the present invention may

include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs. The term “**humanized antibody or antigen-binding fragment thereof**” also includes antibodies and antigen-binding fragment thereof in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. In other words, the term “**humanized antibody or antigen-binding fragment thereof**” may refer to an antibody or antigen-binding fragment thereof in which the CDRs of a recipient human antibody are replaced by CDRs from a donor non-human antibody. Humanized antibodies or antigen-binding fragments thereof may also comprise residues of donor origin in the framework sequences. The humanized antibody or antigen-binding fragment thereof can also comprise at least a portion of a human immunoglobulin constant region. Humanized antibodies or antigen-binding fragments thereof may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. Humanization can be performed using methods known in the art (e.g., Jones *et al.*, **1986**. *Nature*. **321(6069)** :522-5 ; Riechmann *et al.*, **1988**. *Nature*. **332(6162)** :323-7 ; Verhoeven *et al.*, **1988**. *Science*. **239(4847)** :1534-6 ; Presta, **1992**. *Curr Opin Biotechnol*. **3(4)**:394-8; Patent US4,816,567), including techniques such as “superhumanizing” antibodies (e.g., Tan *et al.*, **2002**. *J Immunol*. **169(2)**:1119-25) and “resurfacing” (e.g., Staelens *et al.*, **2006**. *Mol Immunol*. **43(8)**:1243-57; Roguska *et al.*, **1994**. *Proc Natl Acad Sci USA*. **91(3)**:969-73).

[0037] “**Identity**” or “**identical**”, when used herein in a relationship between the sequences of two or more amino acid sequences, or of two or more nucleic acid sequences, refers to the degree of sequence relatedness between amino acid sequences or nucleic acid sequences, as determined by the number of matches between strings of two or more amino acid residues or nucleic acid residues. “Identity” measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*i.e.*, “algorithms”). Identity of related amino acid sequences or nucleic acid sequences can be readily calculated by known methods. Such methods include, but are not limited to, those described in Lesk A. M. (**1988**). *Computational molecular biology: Sources and methods*

for sequence analysis. New York, NY: Oxford University Press; Smith D. W. (1993). *Biocomputing: Informatics and genome projects*. San Diego, CA: Academic Press; Griffin A. M. & Griffin H. G. (1994). *Computer analysis of sequence data, Part 1*. Totowa, NJ: Humana Press; von Heijne G. (1987). *Sequence analysis in molecular biology: treasure trove or trivial pursuit*. San Diego, CA: Academic press; 5 Gribbskov M. R. & Devereux J. (1991). *Sequence analysis primer*. New York, NY: Stockton Press; Carillo *et al.*, 1988. *SIAM J Appl Math.* **48(5)**:1073-82. Preferred methods for determining identity are designed to give the largest match between the sequences tested. Methods of determining identity are described in publicly available computer 10 programs. Preferred computer program methods for determining identity between two sequences include the GCG program package, including GAP (Genetics Computer Group, University of Wisconsin, Madison, WI; Devereux *et al.*, 1984. *Nucleic Acids Res.* **12(1 Pt 1)**:387-95), BLASTP, BLASTN, and FASTA (Altschul *et al.*, 1990. *J Mol Biol.* **215(3)**:403-10). The BLASTX program is publicly available from the National Center 15 for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul *et al.* NCB/NLM/NIH Bethesda, Md. 20894). The well-known Smith Waterman algorithm may also be used to determine identity.

[0038] “**Interleukin-2**” or “**IL-2**” refers to any native IL-2 from any vertebrate source, including mammals such as primates (*e.g.*, humans) and rodents (*e.g.*, mice and rats), 20 unless otherwise indicated. The term encompasses unprocessed IL-2 as well as any form of IL-2 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-2 (*e.g.*, splice variants or allelic variants) as well as truncated forms of IL-2. In one embodiment, IL-2 is human IL-2, having the sequence SEQ ID NO: 1. In another embodiment, IL-2 is a truncated human IL-2, having the sequence SEQ ID 25 NO: 50.

SEQ ID NO: 1

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFCQSIISTLT

30 **SEQ ID NO: 50**

LQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELK
 PLEEVNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNR
 WITFSQSIISTLT

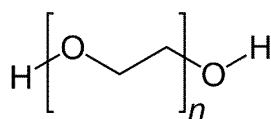
[0039] “**Interleukin-2 receptor**” or “**IL-2R**” refers to the heterotrimeric receptor of IL-
 5 2. This receptor is composed by different combinations of the following chains: α (alpha)
 chain (also called IL-2R α , CD25, or Tac antigen), β (beta) chain (also called IL-2R β , or
 CD122), and γ (gamma) chain (also called IL-2R γ , common gamma chain, or CD132).
 Depending on the combinations of these chains, IL-2 receptors having low, intermediate,
 or high affinity for IL-2 may be generated. Low-affinity IL-2 receptors are composed of
 10 a IL-2R α chain. Intermediate-affinity IL-2 receptors are composed of IL-2R β and IL-2R γ
 chains. High-affinity IL-2 receptors are composed of IL-2R α , IL-2R β and IL-2R γ chains.

[0040] “**IL-2 mutant**” or “**mutant IL-2**” refers to an IL-2 that displays modifications in
 sequence and/or in functional properties.

[0041] “**Mammal**” refers to any mammal, including humans, non-human primates,
 15 domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle,
 horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is a human.

[0042] “**Peptide**” refers to a linear polymer of amino acids of at least 2 amino acids and
 less than 50 amino acids linked together by peptide bonds.

[0043] “**Polyethylene glycol**” or “**PEG**” refers to a biocompatible, synthetic,
 20 hydrophilic polyether compound with the formula hereinabove, wherein $n \geq 4$.



PEG is also known as polyethylene oxide (PEO) or polyoxyethylene (POE), depending
 on its molecular weight. The term “PEG” usually refers to oligomers and polymers with
 a molecular mass below 20,000 g/mol, and “PEO” for polymers with a molecular mass
 25 above 20,000 g/mol. Unless otherwise specified, the term “PEG” as used herein
 comprises both “PEG” and “PEO” compounds.

[0044] “**Single chain antibody**”, as used herein, refers to any antibody or fragment thereof that is a protein having a primary structure comprising or consisting of one uninterrupted sequence of contiguous amino acid residues, including without limitation

5 (1) single-chain Fv molecules (scFv); (2) single chain proteins containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety; and (3) single chain proteins containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light

10 chain moiety.

[0045] “**Single-chain Fv**”, also abbreviated as “**sFv**” or “**scFv**”, refers to antibody fragments that comprise the V_H and V_L antibody domains connected into a single amino acid chain. Preferably, the scFv amino acid sequence further comprises a peptide linker between the V_H and V_L domains that enables the scFv to form the desired structure for

15 antigen binding.

[0046] “**Small organic molecule**” refers to a molecule of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (*e.g.*, proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, more preferably up to 2000 Da, and most preferably

20 up to about 1000 Da.

[0047] “**Subject**”, as used herein, refers to a mammal, preferably a human. In one embodiment, a subject may be a “**patient**”, *i.e.*, a warm-blooded animal, more preferably a human, who/which is awaiting the receipt of, or is receiving medical care or was/is/will be the object of a medical procedure, or is monitored for the development of a disease.

25 [0048] “**Substantially human**”, in the context of the constant region of a humanized or chimeric antibody or antigen-binding fragment thereof, refers to an amino acid sequence identity of at least 70%, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more with a human constant region. The term “human amino acid sequence”, in this context, refers to an amino acid sequence which is encoded by a human

immunoglobulin gene, which includes germline, rearranged and somatically mutated genes. The present invention also contemplates proteins comprising constant domains of “human” sequence which have been altered, by one or more amino acid additions, deletions or substitutions with respect to the human sequence, excepting those
5 embodiments where the presence of a “fully human hinge region” is expressly required. The presence of a “fully human hinge region” in the at least one antibody or antigen-binding fragment thereof comprised in a fusion protein according to the present invention may be beneficial both to minimize immunogenicity and to optimize stability of the antibody. It is considered that one or more amino acid substitutions, insertions or
10 deletions may be made within the constant region of the heavy and/or the light chain, particularly within the Fc region. Amino acid substitutions may result in replacement of the substituted amino acid with a different naturally occurring amino acid, or with a non-natural or modified amino acid. Other structural modifications are also permitted, such as for example changes in glycosylation pattern (*e.g.*, by addition or deletion of *N*- or *O*-
15 linked glycosylation sites).

[0049] “**Therapeutically effective amount**” refers to the level or amount of an antibody as described herein that is aimed at, without causing significant negative or adverse side effects to the target, (1) delaying or preventing the onset of a disease, disorder, or condition; (2) slowing down or stopping the progression, aggravation, or
20 deterioration of one or more symptoms of the disease, disorder, or condition; (3) bringing about ameliorations of the symptoms of the disease, disorder, or condition; (4) reducing the severity or incidence of the disease, disorder, or condition; or (5) curing the disease, disorder, or condition. A therapeutically effective amount may be administered prior to the onset of the disease, disorder, or condition, for a prophylactic or preventive action.
25 Alternatively or additionally, the therapeutically effective amount may be administered after initiation of the disease, disorder, or condition, for a therapeutic action.

[0050] “**Treating**” or “**treatment**” or “**alleviation**” refers to both therapeutic treatment and prophylactic or preventative measures; wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include
30 those already with the disorder as well as those prone to have the disorder or those in

whom the disorder is to be prevented. In one embodiment, a subject is successfully "treated" for a cancer if, after receiving a therapeutic amount of at least one mutant IL-2, fusion protein, nucleic acid or expression vector according to the present invention, the subject shows at least one of the following: reduction in the number of cancer cells (or
5 tumor size) or pathogenic cells; reduction in the percent of total cells that are cancerous or pathogenic; relief to some extent of one or more of the symptoms associated with the cancer or the infectious disease to be treated; reduced morbidity and mortality; and improvement in quality of life issues. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures
10 familiar to a physician.

[0051] "**Unibodies**" refer to an antibody fragment lacking the hinge region of IgG4 antibodies. The deletion of the hinge region results in a molecule that is essentially half the size of traditional IgG4 antibodies and has a univalent binding region rather than the bivalent binding region of IgG4 antibodies.

15 [0052] "**Variant**": refers to nucleic acid or amino acid sequences that typically differs from a nucleic acid or an amino acid sequence specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. When referring to percentage of identity, it is meant a nucleic acid or amino acid sequence comprising at least 70%, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence
20 identity with the reference nucleic acid or amino acid sequence.

DETAILED DESCRIPTION

[0053] This invention relates to mutants of IL-2 comprising at least one transglutaminase site (TG) enabling to conjugate moieties in a simple and reproducible way by
25 transglutamination. However, it will be understood that this concept of adding TG sites to conjugate moieties may be equally applied to other molecules, such as, for example, IL-2 mimetics. Additionally, it will be understood that other types of conjugation via non-natural amino acids and the introduction of other types of coupling sites may be applied at the sites identified within.

[0054] The mutant IL-2 of the invention may exhibit reduced or eliminated affinity for CD25, coupled with modulated affinity for CD122, in which the modulated affinity for CD122 is achieved via transglutaminase-mediated conjugation at one or more single site(s) newly introduced to the IL-2 structure via mutagenesis. The mutant IL-2 may be
5 conjugated to a moiety, such as PEG via a linker which is, or is not, selectively cleaved in diseased tissue. Without willing to be bound to any theory, it is hypothesized that this form of mutant IL-2 may exhibit reduced ability to stimulate T regulatory (Treg) cells (which are CD25-high) while effectively stimulate T effector (Teff) cells (CD25-low). This is a potentially beneficial phenotype in a cancer setting where one would seek to
10 stimulate Teff cells over Treg, thereby stimulating anti-cancer therapeutic activity.

[0055] Alternatively, the mutant IL-2 of the invention may exhibit native (high) affinity for CD25, coupled with modulated affinity for CD122, in which the modulated affinity for CD122 is achieved via transglutaminase-mediated conjugation at one or more single site(s) newly introduced to the IL-2 structure via mutagenesis. The mutant IL-2 may be
15 conjugated to a moiety, such as PEG via a linker which is, or is not, selectively cleaved in diseased tissue. Without willing to be bound to any theory, it is hypothesized that the mutant IL-2 may exhibit enhanced ability to stimulate Treg cells (which are CD25-high) and minimized stimulation of Teff cells (CD25-low). This is a potentially beneficial phenotype in an inflammatory disease setting where one would seek to stimulate Treg
20 cells over Teff, thereby stimulating anti-inflammatory therapeutic activity.

[0056] In one embodiment, the present invention relates to a mutant IL-2, preferably a mutant IL-2 derived from human IL-2.

[0057] In one embodiment, human IL-2 comprises or consists of the sequence with SEQ ID NO: 1 or a variant thereof, preferably wherein said variant comprises at least 70%,
25 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity with SEQ ID NO: 1. Thus, in one embodiment, the mutant IL-2 of the present invention is derived from human IL-2 with SEQ ID NO: 1 or a variant thereof, preferably wherein said variant comprises at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity with SEQ ID NO: 1.

[0058] Said variant may comprise the same number of amino acids as SEQ ID NO: 1, and thus the mutations and positions described herein are the same for the variant. Alternatively, said variant may comprise a different number of amino acids as SEQ ID NO: 1. In this case, the skilled artisan in the art will know how to place the mutations and positions described herein in the variant.

[0059] In one embodiment, the mutant IL-2 comprises a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation), by reference to SEQ ID NO: 1.

[0060] In one embodiment, the mutant IL-2 comprises a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation), by reference to SEQ ID NO: 1.

[0061] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.* F42A mutation), by reference to SEQ ID NO: 1.

[0062] In one embodiment, the IL-2 mutant has a reduced binding or affinity for the IL-2R alpha chain as compared to the IL-2 from which it derives.

[0063] In one embodiment, the IL-2 mutant comprises one or more of the followings modifications:

- a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation), by reference to SEQ ID NO: 1, and/or
- a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation), by reference to SEQ ID NO: 1, and/or
- a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.* F42A mutation), by reference to SEQ ID NO: 1.

[0064] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif.

[0065] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif and a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e. F42A mutation), by reference to SEQ ID NO: 1. Thus, the present invention also relates to a mutant IL-2, preferably derived from the human IL-2 with SEQ ID NO: 1, or a variant thereof, comprising at least one non-endogenous transglutaminase motif, and further comprising a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e. F42A mutation), by reference to SEQ ID NO: 1.

[0066] In one embodiment, the mutant IL-2 comprises one non-endogenous transglutaminase motif. In one embodiment, the mutant IL-2 comprises two or more non-endogenous transglutaminase motifs.

[0067] As used herein, a transglutaminase motif is a motif comprising at least one Q residue, in particular a surface exposed Q residue, whose is recognized by a transglutaminase (*i.e.* the side chain acts as a substrate for the transglutaminase enzyme), which catalyzes the formation of an isopeptide bond between γ -carboxamide groups ($-(C=O)NH_2$) of glutamine residue side chains and various primary amines (acyl-acceptors), notably the ϵ -amino groups ($-NH_2$) of lysine residue side chains, with subsequent release of ammonia (NH_3). Transglutaminase motifs may be found endogenously in proteins, and in particular, in IL-2. As an example, human IL-2 with SEQ ID NO: 1 comprises an endogenous transglutaminase motif with the Q74 residue, and with the motif consisting of AQS.

[0068] As used herein, a non-endogenous transglutaminase motif is a transglutaminase motif that is not found in the IL-2 from which the IL-2 mutant is derived.

[0069] In one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID NO: 1 or a variant thereof, does not comprise non-endogenous transglutaminase motifs that are different from the ones described herein.

[0070] Thus, in one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID NO: 1 or a variant thereof, comprises one or more non-endogenous transglutaminase motif(s) as described herein and does not comprise other non-endogenous transglutaminase motifs.

- 5 [0071] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif inserted within the IL-2 sequence.

[0072] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, Q57,
10 H79, E95, E100, E110 and S130.

[0073] Thus, in one embodiment, the at least one non-endogenous transglutaminase motif is characterized by the presence of a glutamate (Q) residue at one or more of the following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, Q57, H79, E95, E100, E110 and S130.

- 15 [0074] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, Q57, H79, E95, E100, and S130.

[0075] In one embodiment, the at least one non-endogenous transglutaminase motif is
20 inserted by insertion of a glutamate (Q) residue at one or more of the following positions by reference to SEQ ID NO: 1: N33, T41, F42, H79 and E100.

[0076] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by substitution of one or more of the following amino acids by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, H79, E95, E100, E110
25 and S130, with a Q residue.

[0077] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by substitution of one or more of the following amino acids by reference to SEQ

ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, H79, E95, E100, and S130, with a Q residue.

[0078] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by modifying the amino acids surrounding the Q57 residue by reference to SEQ ID NO: 1, in particular, one or more amino acid(s) surrounding the Q57 residue. In this embodiment, the amino acids surrounding the Q57 residue are mutated, preferably substituted by other amino acids, to form a transglutaminase motif.

[0079] Thus, in one embodiment, the at least one non-endogenous transglutaminase motif is inserted by:

- substitution of a Q residue at one or more of the following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, H79, E95, E100, and S130, and/or
- substitution of one or more amino acid(s) surrounding the Q57 residue by reference to SEQ ID NO: 1 to form a transglutaminase motif.

[0080] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by substitution of the amino acid H55 by reference to SEQ ID NO: 1 with a leucine residue (*i.e.* H55L mutation), for the Q57 position.

[0081] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of X_1LQ wherein X_1 is A, L, H, I or V; NX_2Q wherein X_2 is Y, H or F; YRQ, LTQ; AQX_3 wherein X_3 is A or E, TEQ, LFQ; GSQ and VIQ.

[0082] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of X_1LQ wherein X_1 is L, H, I or V; NX_2Q wherein X_2 is Y, H or F; YRQ, LTQR (SEQ ID NO: 2); LFQ; GSQ and VIQ.

[0083] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of LLQ, YRQ, LTQR (SEQ ID NO: 2), LFQ and GSQ.

[0084] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD (SEQ ID NO: 5), MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO: 7), NNYQNP (SEQ ID NO: 8),
5 NNHQNP (SEQ ID NO: 9), LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), LLQC (SEQ ID NO: 13), KNFQLRPRD (SEQ ID NO: 14), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), VLQ, LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ, NNYRQP (SEQ ID NO: 102), NRYRQP (SEQ ID NO: 103), MLTQGF (SEQ ID NO: 104), LAQELK (SEQ ID NO: 105),
10 LAQALK (SEQ ID NO: 106), KATEQK (SEQ ID NO: 107), EYALQ (SEQ ID NO: 108) and DYALQ (SEQ ID NO: 109).

[0085] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD (SEQ ID NO: 5),
15 MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO: 7), NNYQNP (SEQ ID NO: 8), NNHQNP (SEQ ID NO: 9), LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), LLQC (SEQ ID NO: 13), KNFQLRPRD (SEQ ID NO: 14), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), VLQ, LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ, NNYRQP (SEQ ID NO: 102) and
20 NRYRQP (SEQ ID NO: 103).

[0086] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), GSQST (SEQ ID NO: 18), NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103).
25

[0087] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, Q57, H79, E95, E100, E110 and S130, and comprises or consists of a sequence selected from

the group comprising or consisting of X_1LQ wherein X_1 is A, L, H, I or V; NX_2Q wherein X_2 is Y, H or F; YRQ, LTQ; AQX_3 wherein X_3 is A or E; TEQ; LFQ; GSQ and VIQ.

[0088] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions
 5 by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, Q57, H79, E95, E100, E110 and S130, and comprises or consists of a sequence selected from the group comprising or consisting of LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD (SEQ ID NO: 5), MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO: 7), NNYQNP (SEQ ID NO: 8), NNHQNP (SEQ ID NO: 9), LLQFKF (SEQ ID NO:
 10 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), LLQC (SEQ ID NO: 13), KNFQLRPRD (SEQ ID NO: 14), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), VLQ, LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ, NNYRQP (SEQ ID NO: 102), NRYRQP (SEQ ID NO: 103), MLTQGF (SEQ ID NO: 104), LAQELK (SEQ ID NO: 105), LAQALK (SEQ ID NO: 106), KATEQK (SEQ ID
 15 NO: 107), EYALQ (SEQ ID NO: 108) and DYALQ (SEQ ID NO: 109).

[0089] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions
 by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, Q57, H79, E95, E100, and S130, and comprises or consists of a sequence selected from the group
 20 comprising or consisting of X_1LQ wherein X_1 is L, H, I or V; NX_2Q wherein X_2 is Y, H or F; YRQ; LTQR (SEQ ID NO: 2); LFQ; GSQ and VIQ.

[0090] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions
 by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, Q57, H79,
 25 E95, E100, and S130, and comprises or consists of a sequence selected from the group comprising or consisting of LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD (SEQ ID NO: 5), MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO: 7), NNYQNP (SEQ ID NO: 8), NNHQNP (SEQ ID NO: 9), LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), LLQC (SEQ ID NO: 13),
 30 KNFQLRPRD (SEQ ID NO: 14), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ

ID NO: 16), VLQ, LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ, NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103).

[0091] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions
5 by reference to SEQ ID NO: 1: N33, T41, F42, H79 and E100, and comprises or consists of a sequence selected from the group comprising or consisting of LLQ, YRQ, LTQR (SEQ ID NO: 2), LFQ and GSQ.

[0092] In one embodiment, the at least one non-endogenous transglutaminase motif is inserted by insertion of a glutamate (Q) residue at one or more of the following positions
10 by reference to SEQ ID NO: 1: N33, T41, F42, H79 and E100, and comprises or consists of a sequence selected from the group comprising or consisting of LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), GSQST (SEQ ID NO: 18), NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103).

15 [0093] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the L19 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q19 residue, preferably obtained by a L19Q mutation),
20 and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LEHLLQD (SEQ ID NO: 3),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a
25 serine residue (*i.e.*, a C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
30 mutation).

[0094] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 19. An example of such mutant IL-2 is the mutant 2-202.

SEQ ID NO: 19

APTSSSTKKTQLQLEHLLQDLQMILNGINNYKNPKLTRMLTFKFYMPKKATEL
 5 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0095] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 155. An example of such mutant IL-2 is the mutant 2-596.

SEQ ID NO: 155

10 APTSSSTKKTQLQLEHLLQDLQMILNGINNYKNPKLTRMLTFKFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0096] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
 15 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0097] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 130. An example of such mutant IL-2 is the mutant 2-572.

SEQ ID NO: 130

APTSSSTKKTQLQLEHLLQDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
 20 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0098] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- 25 - is inserted by insertion of a Q residue at the L18 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q18 residue, preferably obtained by a L18Q mutation), and

- comprises or consists of the sequence HLQ, preferably comprises or consists of the sequence LEHLQSD (SEQ ID NO: 4),
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0099] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 20. An example of such mutant IL-2 is the mutant 2-203.

SEQ ID NO: 20

APTSSSTKKTQLQLEHLQSDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0100] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 156. An example of such mutant IL-2 is the mutant 2-597.

SEQ ID NO: 156

APTSSSTKKTQLQLEHLQSDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0101] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

[0102] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 131. An example of such mutant IL-2 is the mutant 2-573.

SEQ ID NO: 131

APTSSSTKKTQLQLEHLQSDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0103] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
 5 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
 motif:

- is inserted by insertion of a Q residue at the H16 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q16 residue, preferably obtained by a H16Q mutation),
 and
- 10 - comprises or consists of the sequence LLQ, preferably comprises or consists of
 the sequence LLQLLLD (SEQ ID NO: 5),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
 15 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
 both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 mutation).

20 [0104] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 21. An example of such mutant IL-2 is the mutant 2-204.

SEQ ID NO: 21

APTSSSTKKTQLQLLQLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 25 ETATIVEFLNRWITFSQSIISTLT

[0105] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 157. An example of such mutant IL-2 is the mutant 2-598.

SEQ ID NO: 157

APTSSSTKKTQLQLQLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0106] In one embodiment, the mutant IL-2 comprises a substitution of the
5 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
(i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0107] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 132. An example of such mutant IL-2 is the mutant 2-574.

SEQ ID NO: 132

10 APTSSSTKKTQLQLQLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0108] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
15 motif:

- is inserted by insertion of a Q residue at the N26 position by reference to SEQ ID
NO: 1 (*i.e.* comprises a Q26 residue, preferably obtained by a N26Q mutation),
and
- comprises or consists of the sequence ILQ, preferably comprises or consists of the
20 sequence MILQGIN (SEQ ID NO: 6),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
substitution of the cysteine residue 125 with any other amino acid residue, preferably a
serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
25 both a substitution of the cysteine residue 125 with any other amino acid residue,
preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
mutation).

[0109] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 22. An example of such mutant IL-2 is the mutant 2-205.

SEQ ID NO: 22

APTSSSTKKTQLQLEHLLLDLQMILQGINNYKNPKLTRMLTFKFYMPKKATELK
5 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0110] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 158. An example of such mutant IL-2 is the mutant 2-599.

SEQ ID NO: 158

10 APTSSSTKKTQLQLEHLLLDLQMILQGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0111] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
15 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0112] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 133. An example of such mutant IL-2 is the mutant 2-575.

SEQ ID NO: 133

APTSSSTKKTQLQLEHLLLDLQMILQGINNYKNPKLTRMLTAKFYMPKKATEL
20 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0113] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- 25 - is inserted by insertion of a Q residue at the N26 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q26 residue, preferably obtained by a N26Q mutation), and

- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence MLLQGIN (SEQ ID NO: 7),
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0114] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 23. An example of such mutant IL-2 is the mutant 2-206.

SEQ ID NO: 23

APTSSSTKKTQLQLEHLLLDLQMLLQGINNYKNPKLTRMLTFKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0115] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 159. An example of such mutant IL-2 is the mutant 2-600.

SEQ ID NO: 159

APTSSSTKKTQLQLEHLLLDLQMLLQGINNYKNPKLTRMLTFKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

- [0116] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

[0117] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 134. An example of such mutant IL-2 is the mutant 2-576.

SEQ ID NO: 134

APTSSSTKKTQLQLEHLLLDLQMLLQGINNYKNPKLTRMLTFKAYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0118] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
 5 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
 motif:

- is inserted by insertion of a Q residue at the K32 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q32 residue, preferably obtained by a K32Q mutation),
 and
- 10 - comprises or consists of the sequence NYQ, preferably comprises or consists of
 the sequence NNYQNP (SEQ ID NO: 8),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.* C125S mutation), (ii) a substitution of the glutamine 74 residue with
 15 any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii)
 both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 mutation).

20 [0119] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 24. An example of such mutant IL-2 is the mutant 2-207.

SEQ ID NO: 24

APTSSSTKKTQLQLEHLLLDLQMILNGINNYQNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 25 ETATIVEFLNRWITFSQSIISTLT

[0120] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 160. An example of such mutant IL-2 is the mutant 2-601.

SEQ ID NO: 160

APTSSSTKKTQLQLEHLLLDLQMILNGINNYQNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0121] In one embodiment, the mutant IL-2 comprises a substitution of the
5 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
(i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0122] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 135. An example of such mutant IL-2 is the mutant 2-577.

SEQ ID NO: 135

10 APTSSSTKKTQLQLEHLLLDLQMILNGINNYQNPKLTRMLTAKFYMPKKATEL
KHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0123] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
15 motif:

- is inserted by insertion of a Q residue at the K32 position by reference to SEQ ID
NO: 1 (*i.e.* comprises a Q32 residue, preferably obtained by a K32Q mutation),
and
- comprises or consists of the sequence NHQ, preferably comprises or consists of
20 the sequence NNHQNP (SEQ ID NO: 9),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
substitution of the cysteine residue 125 with any other amino acid residue, preferably a
serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii)
25 both a substitution of the cysteine residue 125 with any other amino acid residue,
preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
mutation).

[0124] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 25. An example of such mutant IL-2 is the mutant 2-208.

SEQ ID NO: 25

APTSSSTKKTQLQLEHLLLDLQMILNGINNHQNPCLTRMLTFKIFYMPKKATELK
 5 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0125] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 161. An example of such mutant IL-2 is the mutant 2-602.

SEQ ID NO: 161

10 APTSSSTKKTQLQLEHLLLDLQMILNGINNHQNPCLTRMLTFKIFYMPKKATELK
 HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0126] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
 15 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0127] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 136. An example of such mutant IL-2 is the mutant 2-578.

SEQ ID NO: 136

APTSSSTKKTQLQLEHLLLDLQMILNGINNHQNPCLTRMLTAKIFYMPKKATEL
 20 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0128] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- 25 - is inserted by insertion of a Q residue at the N33 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q33 residue, preferably obtained by a N33Q mutation), and

- comprises or consists of the sequence YRQ, preferably comprises or consists of the sequence NNYRQP (SEQ ID NO: 102),
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.* C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0129] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 110. An example of such mutant IL-2 is the mutant 2-103.

SEQ ID NO: 110

APTSSSTKKTQLQLEHLLLDLQMILNGINNYRQPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0130] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 162. An example of such mutant IL-2 is the mutant 2-603.

SEQ ID NO: 162

APTSSSTKKTQLQLEHLLLDLQMILNGINNYRQPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0131] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

[0132] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 137. An example of such mutant IL-2 is the mutant 2-579.

SEQ ID NO: 137

APTSSSTKKTQLQLEHLLLDLQMILNGINNYRQPKLTRMLTAKFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0133] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
 5 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
 motif:

- is inserted by insertion of a Q residue at the N33 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q33 residue, preferably obtained by a N33Q mutation),
 and
- 10 - comprises or consists of the sequence YRQ, preferably comprises or consists of
 the sequence NRYRQP (SEQ ID NO: 103),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.* C125S mutation), (ii) a substitution of the glutamine 74 residue with
 15 any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii)
 both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 mutation).

20 [0134] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 111. An example of such mutant IL-2 is the mutant 2-104.

SEQ ID NO: 111

APTSSSTKKTQLQLEHLLLDLQMILNGINRYRQPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 25 ETATIVEFLNRWITFSQSIISTLT

[0135] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 163. An example of such mutant IL-2 is the mutant 2-604.

SEQ ID NO: 163

APTSSSTKKTQLQLEHLLLDLQMILNGINRYRQPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0136] In one embodiment, the mutant IL-2 comprises a substitution of the
 5 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0137] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 138. An example of such mutant IL-2 is the mutant 2-580.

SEQ ID NO: 138

10 APTSSSTKKTQLQLEHLLLDLQMILNGINRYRQPKLTRMLTAKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0138] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
 15 motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation),
 and
- comprises or consists of the sequence LLQ, preferably comprises or consists of
 20 the sequence preferably LLQFKF (SEQ ID NO: 10),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
 25 both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 mutation).

[0139] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 26. An example of such mutant IL-2 is the mutant 2-105.

SEQ ID NO: 26

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
5 HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0140] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 164. An example of such mutant IL-2 is the mutant 2-605.

SEQ ID NO: 164

10 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0141] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
15 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0142] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 139. An example of such mutant IL-2 is the mutant 2-581.

SEQ ID NO: 139

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
20 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0143] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- 25 - is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and

- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11),
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.* C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0144] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 27. An example of such mutant IL-2 is the mutant 2-106.

SEQ ID NO: 27

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0145] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 62. An example of such mutant IL-2 is the mutant 2-130.

SEQ ID NO: 62

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0146] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and

- comprises or consists of the sequence LTQ, in particular LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0147] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 28. An example of such mutant IL-2 is the mutant 2-107.

SEQ ID NO: 28

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0148] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 63. An example of such mutant IL-2 is the mutant 2-131.

SEQ ID NO: 63

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0149] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and

- comprises or consists of the sequence LTQ, in particular LTQG (SEQ ID NO: 118), preferably comprises or consists of the sequence MLTQGF (SEQ ID NO: 104),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0150] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 112. An example of such mutant IL-2 is the mutant 2-108.

SEQ ID NO: 112

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQGFYMPKKATEL
KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0151] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 165. An example of such mutant IL-2 is the mutant 2-606.

SEQ ID NO: 165

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQGFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0152] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the T51 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q51 residue, preferably obtained by a T51Q mutation), and

- comprises or consists of the sequence AQE, preferably comprises or consists of the sequence LAQELK (SEQ ID NO: 105),
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0153] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 113. An example of such mutant IL-2 is the mutant 2-109.

SEQ ID NO: 113

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFK FYMPKLAQELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0154] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 166. An example of such mutant IL-2 is the mutant 2-607.

SEQ ID NO: 166

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFK FYMPKLAQELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0155] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

[0156] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 140. An example of such mutant IL-2 is the mutant 2-582.

SEQ ID NO: 140

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKLAQEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0157] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
 5 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
 motif:

- is inserted by insertion of a Q residue at the T51 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q51 residue, preferably obtained by a T51Q mutation),
 and
- 10 - comprises or consists of the sequence AQA, preferably comprises or consists of
 the sequence LAQALK (SEQ ID NO: 106),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
 15 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
 both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 mutation).

20 [0158] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 114. An example of such mutant IL-2 is the mutant 2-110.

SEQ ID NO: 114

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKLAQAL
 KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 25 DETATIVEFLNRWITFSQSIISTLT

[0159] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 167. An example of such mutant IL-2 is the mutant 2-608.

SEQ ID NO: 167

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKLAQAL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0160] In one embodiment, the mutant IL-2 comprises a substitution of the
 5 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0161] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 141. An example of such mutant IL-2 is the mutant 2-583.

SEQ ID NO: 141

10 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKLAQAL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0162] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
 15 motif:

- is inserted by insertion of a Q residue at the L53 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q53 residue, preferably obtained by a L53Q mutation),
 and
- comprises or consists of the sequence TEQ, preferably comprises or consists of
 20 the sequence KATEQK (SEQ ID NO: 107),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
 25 both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 mutation).

[0163] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 115. An example of such mutant IL-2 is the mutant 2-111.

SEQ ID NO: 115

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATEQ
5 KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0164] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 168. An example of such mutant IL-2 is the mutant 2-609.

SEQ ID NO: 168

10 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATEQ
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0165] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
15 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0166] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 142. An example of such mutant IL-2 is the mutant 2-584.

SEQ ID NO: 142

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEQ
20 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0167] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- 25
- is inserted by insertion of a Q residue at the Q57 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q57 residue), and
 - comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQC (SEQ ID NO: 13),

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
- 5 both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

- [0168] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
- 10 SEQ ID NO: 29. An example of such mutant IL-2 is the mutant 2-209.

SEQ ID NO: 29

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
LLQCLEEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

- 15 [0169] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 169. An example of such mutant IL-2 is the mutant 2-610.

SEQ ID NO: 169

- APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
LLQCLEEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
- 20 ETATIVEFLNRWITFSQSIISTLT

[0170] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

- [0171] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
- 25 SEQ ID NO: 143. An example of such mutant IL-2 is the mutant 2-585.

SEQ ID NO: 143

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KLLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0172] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
5 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID
NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation),
and
- 10 - comprises or consists of the sequence NFQ, preferably comprises or consists of
the sequence KNFQLRPRD (SEQ ID NO: 14),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
substitution of the cysteine residue 125 with any other amino acid residue, preferably a
serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
15 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
both a substitution of the cysteine residue 125 with any other amino acid residue,
preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
mutation).

20 [0173] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 30. An example of such mutant IL-2 is the mutant 2-210.

SEQ ID NO: 30

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFQLRPRDLISNINVIVLELKGSETTFMCEYAD
25 ETATIVEFLNRWITFSQSIISTLT

[0174] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 170. An example of such mutant IL-2 is the mutant 2-611.

SEQ ID NO: 170

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFQLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0175] In one embodiment, the mutant IL-2 comprises a substitution of the
5 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
(i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0176] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 144. An example of such mutant IL-2 is the mutant 2-222.

SEQ ID NO: 144

10 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEELKPLEEVLNLAESKNFQLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0177] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
15 motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID
NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation),
and
- comprises or consists of the sequence LFQ, preferably comprises or consists of
20 the sequence KLFQLRPRD (SEQ ID NO: 15),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
substitution of the cysteine residue 125 with any other amino acid residue, preferably a
serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
25 both a substitution of the cysteine residue 125 with any other amino acid residue,
preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
mutation).

[0178] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 31. An example of such mutant IL-2 is the mutant 2-112.

SEQ ID NO: 31

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 5 HLQCLEEELKPLEEVLNLAQSKLFQLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0179] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 32. An example of such mutant IL-2 is the mutant 2-114.

SEQ ID NO: 32

10 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKLFQLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0180] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
 15 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0181] Thus, in one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID
 20 NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation),
 and
- comprises or consists of the sequence LFQ, preferably comprises or consists of
 the sequence KLFQLRPRD (SEQ ID NO: 15), and

wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with
 25 any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by
 reference to SEQ ID NO: 1,

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)
 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation),

or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

- 5 [0182] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 64. An example of such mutant IL-2 is the mutant 2-132.

SEQ ID NO: 64

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKLFQLRPRDLISNINVIVLELKGSETTFMCEYA
10 DETATIVEFLNRWITFSQSIISTLT

[0183] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID
15 NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation),
and
- comprises or consists of the sequence LLQ, preferably comprises or consists of
the sequence KLLQLRPRD (SEQ ID NO: 16),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
20 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
both a substitution of the cysteine residue 125 with any other amino acid residue,
preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
25 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
mutation).

[0184] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 33. An example of such mutant IL-2 is the mutant 2-113.

SEQ ID NO: 33

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAQSKLLQLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

- [0185] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 5 SEQ ID NO: 34. An example of such mutant IL-2 is the mutant 2-115.

SEQ ID NO: 34

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKLLQLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

- 10 [0186] In one embodiment, the mutant IL-2 comprises a substitution of the
 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

- [0187] Thus, in one embodiment, the mutant IL-2 comprises at least one non-
 endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof,
 15 wherein said motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID
 NO: 1 (i.e., comprises a Q79 residue, preferably obtained by a H79Q mutation),
 and
- comprises or consists of the sequence LLQ, preferably comprises or consists of
 20 the sequence KLLQLRPRD (SEQ ID NO: 16), and

wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with
 any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by
 reference to SEQ ID NO: 1,

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 25 substitution of the cysteine residue 125 with a serine residue (i.e., C125S mutation), (ii)
 a substitution of the glutamine 74 residue with a glutamate residue (i.e., Q74E mutation),
 or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (i.e., a C125S mutation) and a substitution of the glutamine 74

residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0188] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 65. An example of such mutant IL-2 is the mutant 2-133.

5 SEQ ID NO: 65

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKLLQLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0189] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
10 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the E95 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q95 residue, preferably obtained by a E95Q mutation), and
- 15 - comprises or consists of the sequence VLQ,
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
20 both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0190] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
25 SEQ ID NO: 35. An example of such mutant IL-2 is the mutant 2-211.

SEQ ID NO: 35

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLQLKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0191] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 171. An example of such mutant IL-2 is the mutant 2-612.

SEQ ID NO: 171

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 5 HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLQLKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0192] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

10 [0193] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 145. An example of such mutant IL-2 is the mutant 2-586.

SEQ ID NO: 145

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLQLKGSETTFMCEYA
 15 DETATIVEFLNRWITFSQSIISTLT

[0194] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the E95 position by reference to SEQ ID
 20 NO: 1 (*i.e.* comprises a Q95 residue, preferably obtained by a E95Q mutation),
 and

- comprises or consists of the sequence LLQ,
 optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 25 serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
 any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii)
 both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74

residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0195] In one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID NO: 1 or a variant thereof comprises or consists of the sequence with SEQ ID NO: 36.

- 5 An example of such mutant IL-2 is the mutant 2-212.

SEQ ID NO: 36

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVILLQLKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

- 10 [0196] In one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID NO: 1 or a variant thereof comprises or consists of the sequence with SEQ ID NO: 172.
An example of such mutant IL-2 is the mutant 2-613.

SEQ ID NO: 172

- 15 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVILLQLKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0197] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

- 20 [0198] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 146. An example of such mutant IL-2 is the mutant 2-587.

SEQ ID NO: 146

- 25 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVILLQLKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0199] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the E100 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q100 residue, preferably obtained by a E100Q mutation), and
- comprises or consists of the sequence GSQ, preferably comprises or consists of the sequence GSQTT (SEQ ID NO: 17),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0200] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 37. An example of such mutant IL-2 is the mutant 2-213.

SEQ ID NO: 37

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSQTTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0201] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 173. An example of such mutant IL-2 is the mutant 2-614.

SEQ ID NO: 173

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSQTTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0202] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0203] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
5 SEQ ID NO: 147. An example of such mutant IL-2 is the mutant 2-588.

SEQ ID NO: 147

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSQTTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

10 [0204] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the E100 position by reference to SEQ ID NO: 1 (i.e. comprises a Q100 residue, preferably obtained by a E100Q mutation), and
- 15 - comprises or consists of the sequence GSQ, preferably comprises or consists of the sequence GSQST (SEQ ID NO: 18),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a
20 serine residue (i.e., C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (i.e., a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., a Q74E
25 mutation).

[0205] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 38. An example of such mutant IL-2 is the mutant 2-116.

SEQ ID NO: 38

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSQSTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

- [0206] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 5 SEQ ID NO: 174. An example of such mutant IL-2 is the mutant 2-615.

SEQ ID NO: 174

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSQSTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

- 10 [0207] In one embodiment, the mutant IL-2 comprises a substitution of the
 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

- [0208] Thus, in one embodiment, the mutant IL-2 comprises at least one non-
 endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof,
 15 wherein said motif:

- is inserted by insertion of a Q residue at the E100 position by reference to SEQ
 ID NO: 1 (i.e., comprises a Q100 residue, preferably obtained by a E100Q
 mutation), and
- comprises or consists of the sequence GSQ, preferably comprises or consists of
 20 the sequence GSQST (SEQ ID NO: 18), and

wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with
 any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by
 reference to SEQ ID NO: 1,

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 25 substitution of the cysteine residue 125 with a serine residue (i.e., C125S mutation), (ii)
 a substitution of the glutamine 74 residue with a glutamate residue (i.e., Q74E mutation),
 or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (i.e., a C125S mutation) and a substitution of the glutamine 74

residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0209] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 66. An example of such mutant IL-2 is the mutant 2-134.

5 SEQ ID NO: 66

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSQSTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0210] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
10 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the E110 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q110 residue, preferably obtained by a E110Q mutation), and
- 15 - comprises or consists of the sequence ALQ, preferably comprises or consists of the sequence EYALQ (SEQ ID NO: 108),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
20 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

25 [0211] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 116. An example of such mutant IL-2 is the mutant 2-117.

SEQ ID NO: 116

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAL
QTATIVEFLNRWITFSQSIISTLT

- [0212] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
5 SEQ ID NO: 175. An example of such mutant IL-2 is the mutant 2-616.

SEQ ID NO: 175

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAL
QTATIVEFLNRWITFSQSIISTLT

- 10 [0213] In one embodiment, the mutant IL-2 comprises a substitution of the
phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
(i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0214] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 148. An example of such mutant IL-2 is the mutant 2-589.

- 15 SEQ ID NO: 148

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
LQTATIVEFLNRWITFSQSIISTLT

- [0215] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
20 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
motif:

- is inserted by insertion of a Q residue at the E110 position by reference to SEQ
ID NO: 1 (i.e. comprises a Q110 residue, preferably obtained by a E110Q
mutation), and
- 25 - comprises or consists of the sequence ALQ, preferably comprises or consists of
the sequence DYALQ (SEQ ID NO: 109),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
substitution of the cysteine residue 125 with any other amino acid residue, preferably a

- serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0216] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 117. An example of such mutant IL-2 is the mutant 2-118.

SEQ ID NO: 117

- 10 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCDYAL
QTATIVEFLNRWITFSQSIISTLT

[0217] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 176. An example of such mutant IL-2 is the mutant 2-617.

- 15 SEQ ID NO: 176

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCDYAL
QTATIVEFLNRWITFSQSIISTLT

- 20 [0218] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

[0219] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 149. An example of such mutant IL-2 is the mutant 2-590.

SEQ ID NO: 149

- 25 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCDYA
LQTATIVEFLNRWITFSQSIISTLT

[0220] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a Q residue at the S130 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q130 residue, preferably obtained by a S130Q mutation), and
 - comprises or consists of the sequence VIQ,
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0221] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 39. An example of such mutant IL-2 is the mutant 2-214.

SEQ ID NO: 39

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSVIQTLT

[0222] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 177. An example of such mutant IL-2 is the mutant 2-618.

SEQ ID NO: 177

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSVIQTLT

[0223] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0224] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
5 SEQ ID NO: 150. An example of such mutant IL-2 is the mutant 2-591.

SEQ ID NO: 150

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSVIQTLT

10 [0225] In one embodiment, the mutant IL-2 of the present invention, preferably derived from human IL-2 with SEQ ID NO: 1 or a variant thereof, comprises at least one non-endogenous transglutaminase motif that is inserted at the N-terminus or C-terminus of the IL-2 sequence.

[0226] In one embodiment, the at least one non-endogenous transglutaminase motif
15 comprises or consists of a sequence selected from the group comprising or consisting of LQS and TQG, preferably wherein the sequence is present or added at the N- or C-terminus.

[0227] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of
20 LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), TQGAPTSSSTKKTQ (SEQ ID NO: 41), LQSPTSSSTKKTQ (SEQ ID NO: 42), TQGASSSTKKTQ (SEQ ID NO: 43), TQGASSTKKTQ (SEQ ID NO: 44), TQGASTKKTQ (SEQ ID NO: 45), GGTQGA (SEQ ID NO: 46), GGLQSP (SEQ ID NO: 47), GLQSP (SEQ ID NO: 48), LQSP (SEQ ID NO: 49), and QGA, preferably wherein said sequence is present or added at the N- or
25 C-terminus.

[0228] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), TQGAPTSSSTKKTQ (SEQ ID NO: 41),

LQSPTSSSTKKTQ (SEQ ID NO: 42), TQGASSSTKKTQ (SEQ ID NO: 43), TQGASSTKKTQ (SEQ ID NO: 44), and TQGASTKKTQ (SEQ ID NO: 45), preferably wherein said sequence is present or added at the N-terminus.

[0229] In one embodiment, the at least one non-endogenous transglutaminase motif
5 comprises or consists of a sequence selected from the group comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), LQSPTSSSTKKTQ (SEQ ID NO: 42), and TQGASSTKKTQ (SEQ ID NO: 44), preferably wherein said sequence is present or added at the N-terminus.

[0230] In one embodiment, the at least one non-endogenous transglutaminase motif
10 defined hereinabove is inserted at the N-terminus of an IL-2 sequence with SEQ ID NO: 50.

[0231] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of GGTQGA (SEQ ID NO: 46), GGLQSP (SEQ ID NO: 47), GLQSP (SEQ ID NO: 48),
15 LQSP (SEQ ID NO: 49) and QGA, preferably wherein said sequence is present or added at the C-terminus.

[0232] In one embodiment, the at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected from the group comprising or consisting of GGLQSP (SEQ ID NO: 47), GLQSP (SEQ ID NO: 48), LQSP (SEQ ID NO: 49) and
20 QGA, preferably wherein said sequence is present or added at the C-terminus.

[0233] In one embodiment, the at least one non-endogenous transglutaminase motif defined hereinabove is inserted at the C-terminus of the IL-2 sequence with SEQ ID NO: 1.

[0234] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
25 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of the sequence LQSPGAPTSSSTKKTQ

- (SEQ ID NO: 40), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), at the N-terminus),
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
- 5 substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
- 10 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0235] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), is inserted at the N-terminus of the sequence with SEQ ID NO: 1.

- 15 [0236] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0237] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 51. An example of such mutant IL-2 is the mutant 2-120.

- 20 SEQ ID NO: 51
 LQSPGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKK
 ATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFM
 CEYADETATIVEFLNRWITFSQSIISTLT

- [0238] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
- 25 SEQ ID NO: 178. An example of such mutant IL-2 is the mutant 2-619.

SEQ ID NO: 178

LQSPGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKK
ATELKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFM
CEYADETATIVEFLNRWITFSQSIISTLT

[0239] In one embodiment, the mutant IL-2 comprises a substitution of the
5 phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
(i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0240] Thus, in one embodiment, the mutant IL-2 comprises at least one non-
endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof,
wherein said motif:

10 - is inserted by insertion of a sequence comprising or consisting of LQS, preferably
a sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO:
40), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS,
preferably a sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ
ID NO: 40), at the N-terminus), and

15 wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with
any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by
reference to SEQ ID NO: 1,

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)
20 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation),
or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue,
preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
mutation).

25 [0241] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 67. An example of such mutant IL-2 is the mutant 2-135.

SEQ ID NO: 67

LQSPGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKK
ATELKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFM
CEYADETATIVEFLNRWITFSQSIISTLT

[0242] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
5 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
motif:

- is inserted by insertion of a sequence comprising or consisting of TQG, preferably
a sequence comprising or consisting of TQGAPTSSSTKKTQ (SEQ ID NO: 41),
at the N-terminus (*i.e.* comprises a sequence comprising or consisting of TQG,
10 preferably a sequence comprising or consisting of TQGAPTSSSTKKTQ (SEQ
ID NO: 41), at the N-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
substitution of the cysteine residue 125 with any other amino acid residue, preferably a
serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
15 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
both a substitution of the cysteine residue 125 with any other amino acid residue,
preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
mutation).

20 [0243] In one embodiment, the sequence comprising or consisting of TQG, preferably
the sequence comprising or consisting of TQGAPTSSSTKKTQ (SEQ ID NO: 41), is
inserted at the N-terminus of the sequence with SEQ ID NO: 1.

[0244] In one embodiment, the sequence comprising or consisting of TQG, preferably
the sequence comprising or consisting of TQGAPTSSSTKKTQ (SEQ ID NO: 41), is
25 inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0245] In one embodiment, the mutant IL-2 thereof comprises or consists of the
sequence with SEQ ID NO: 52. An example of such mutant IL-2 is the mutant 2-215.

SEQ ID NO: 52

TQGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA
 TELKHLQCLEEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCE
 YADETATIVEFLNRWITFSQSIISTLT

- 5 [0246] In one embodiment, the mutant IL-2 thereof comprises or consists of the sequence with SEQ ID NO: 179. An example of such mutant IL-2 is the mutant 2-620.

SEQ ID NO: 179

TQGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA
 TELKHLQCLEEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC
 EYADETATIVEFLNRWITFSQSIISTLT

- 10 [0247] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0248] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 151. An example of such mutant IL-2 is the mutant 2-592.

- 15 SEQ ID NO: 151

TQGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKA
 TELKHLQCLEEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCE
 YADETATIVEFLNRWITFSQSIISTLT

- 20 [0249] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus (i.e. comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus),
- 25

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a

- serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0250] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), is inserted at the N-terminus of the sequence with SEQ ID NO: 1.

- 10 [0251] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0252] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 53. An example of such mutant IL-2 is the mutant 2-121.

- 15 SEQ ID NO: 53

LQSPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATE
LKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

- [0253] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
20 SEQ ID NO: 180. An example of such mutant IL-2 is the mutant 2-621.

SEQ ID NO: 180

LQSPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATE
LKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

- 25 [0254] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

[0255] Thus, in one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus (*i.e.*, comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus), and

wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1,

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0256] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 68. An example of such mutant IL-2 is the mutant 2-136.

SEQ ID NO: 68

LQSPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATE
LKHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

[0257] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSSTKKTQ (SEQ ID NO: 43), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of TQG,

preferably a sequence comprising or consisting of TQGASSSTKKTQ (SEQ ID NO: 43), at the N-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0258] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASSSTKKTQ (SEQ ID NO: 43), is inserted at the N-terminus of the sequence with SEQ ID NO: 1.

[0259] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASSSTKKTQ (SEQ ID NO: 43), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0260] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 54. An example of such mutant IL-2 is the mutant 2-216.

SEQ ID NO: 54

20 TQGASSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKIFYMPKKATE
LKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

[0261] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 181. An example of such mutant IL-2 is the mutant 2-622.

25 SEQ ID NO: 181

TQGASSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKIFYMPKKATE
LKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

[0262] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0263] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
5 SEQ ID NO: 152. An example of such mutant IL-2 is the mutant 2-593.

SEQ ID NO: 152

TQGASSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTAKFYMPKKATE
LKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

10 [0264] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at
15 the N-terminus (i.e. comprises a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a
20 serine residue (i.e., C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (i.e., a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., a Q74E
25 mutation).

[0265] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), is inserted at the N-terminus of the sequence with SEQ ID NO: 1.

[0266] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0267] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
5 SEQ ID NO: 55. An example of such mutant IL-2 is the mutant 2-122.

SEQ ID NO: 55

TQGASSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

10 [0268] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
SEQ ID NO: 182. An example of such mutant IL-2 is the mutant 2-623.

SEQ ID NO: 182

TQGASSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
15 DETATIVEFLNRWITFSQSIISTLT

[0269] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0270] Thus, in one embodiment, the mutant IL-2 comprises at least one non-
20 endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID
25 NO: 44), at the N-terminus), and

wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1,

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 5 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)
 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation),
 or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 10 mutation).

[0271] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 69. An example of such mutant IL-2 is the mutant 2-137.

SEQ ID NO: 69

TQGASSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
 15 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0272] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- 20 - is inserted by insertion of a sequence comprising or consisting of TQG, preferably
 a sequence comprising or consisting of TQGASTKKTQ (SEQ ID NO: 45), at the
 N-terminus (*i.e.* comprises a sequence comprising or consisting of TQG,
 preferably a sequence comprising or consisting of TQGASTKKTQ (SEQ ID NO:
 45), at the N-terminus),
 25 optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.* C125S mutation), (ii) a substitution of the glutamine 74 residue with
 any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation), or (iii)
 both a substitution of the cysteine residue 125 with any other amino acid residue,
 30 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74

residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0273] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASTKKTQ (SEQ ID NO: 45), is inserted
5 at the N-terminus of the sequence with SEQ ID NO: 1.

[0274] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASTKKTQ (SEQ ID NO: 45), is inserted at the N-terminus of the sequence with SEQ ID NO: 50. An example of such mutant IL-2 is the mutant 2-217.

10 [0275] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 56. An example of such mutant IL-2 is the mutant 2-217.

SEQ ID NO: 56

TQGASTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
15 ETATIVEFLNRWITFSQSIISTLT

[0276] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 183. An example of such mutant IL-2 is the mutant 2-624.

SEQ ID NO: 183

TQGASTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
20 HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0277] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

25 [0278] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 153. An example of such mutant IL-2 is the mutant 2-594.

SEQ ID NO: 153

TQGASTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

5 [0279] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of GGTQGA (SEQ ID NO: 46), at the C-terminus (*i.e.* comprises a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of GGTQGA (SEQ ID NO: 46), at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), and/or a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0280] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of GGTQGA (SEQ ID NO: 46), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0281] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 57. An example of such mutant IL-2 is the mutant 2-218.

SEQ ID NO: 57

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTGGTQGA

[0282] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 184. An example of such mutant IL-2 is the mutant 2-625.

SEQ ID NO: 184

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 5 HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLTGGTQGA

[0283] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

10 [0284] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 154. An example of such mutant IL-2 is the mutant 2-595.

SEQ ID NO: 154

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 15 DETATIVEFLNRWITFSQSIISTLTGGTQGA

[0285] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably
 20 a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus (i.e. comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 25 substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (i.e., C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (i.e., a C125S mutation) and a substitution of the glutamine 74

residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0286] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), is inserted at the
5 C-terminus of the sequence with SEQ ID NO: 1.

[0287] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), is inserted at the C-terminus of the sequence with SEQ ID NO: 50.

[0288] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
10 SEQ ID NO: 58. An example of such mutant IL-2 is the mutant 2-123.

SEQ ID NO: 58

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTGGLQSP

[0289] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
15 SEQ ID NO: 185. An example of such mutant IL-2 is the mutant 2-626.

SEQ ID NO: 185

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
20 ETATIVEFLNRWITFSQSIISTLTGGLQSP

[0290] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

[0291] Thus, in one embodiment, the mutant IL-2 comprises at least one non-
25 endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus (*i.e.*, comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus), and
- 5 wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1,
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
- 10 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
- 15 mutation).

[0292] In one embodiment, the mutant IL- comprises or consists of the sequence with SEQ ID NO: 70. An example of such mutant IL-2 is the mutant 2-138.

SEQ ID NO: 70
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
20 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLTGGLQSP

[0293] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- 25
- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48) at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48), at the C-terminus),

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii)
- 5 both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

- [0294] In one embodiment, the sequence comprising or consisting of LQS, preferably
- 10 the sequence comprising or consisting of GLQSP (SEQ ID NO: 48), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0295] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GLQSP (SEQ ID NO: 48), is inserted at the C-terminus of the sequence with SEQ ID NO: 50.

- 15 [0296] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 59. An example of such mutant IL-2 is the mutant 2-124.

SEQ ID NO: 59

- APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
- 20 ETATIVEFLNRWITFSQSIISTLTGLQSP

[0297] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 186. An example of such mutant IL-2 is the mutant 2-627.

SEQ ID NO: 186

- APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
- 25 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
- ETATIVEFLNRWITFSQSIISTLTGLQSP

[0298] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0299] Thus, in one embodiment, the mutant IL-2 comprises at least one non-
5 endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48) at the C-terminus (i.e., comprises a sequence comprising or consisting of LQS, preferably
10 a sequence comprising or consisting of GLQSP (SEQ ID NO: 48), at the C-terminus),

wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1,

- 15 optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (i.e., C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (i.e., Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (i.e., a C125S mutation) and a substitution of the glutamine 74
20 residue with any other amino acid residue, preferably a glutamate residue (i.e., a Q74E mutation).

[0300] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 122. An example of such mutant IL-2 is the mutant 2-139.

SEQ ID NO: 122

25 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLTGLQSP

[0301] In one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting LQSP (SEQ ID NO: 49), at the C-terminus), optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0302] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSP (SEQ ID NO: 49), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0303] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSP (SEQ ID NO: 49), is inserted at the C-terminus of the sequence with SEQ ID NO: 50.

[0304] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 60. An example of such mutant IL-2 is the mutant 2-125.

SEQ ID NO: 60

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTQSP

[0305] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 187. An example of such mutant IL-2 is the mutant 2-628.

SEQ ID NO: 187

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEELKPLEEVNLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTQLQSP

- 5 [0306] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0307] Thus, in one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof,
10 wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus (i.e. comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus), and

15 wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (i.e., F42A mutation), by reference to SEQ ID NO: 1,

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (i.e., C125S mutation), (ii)
20 a substitution of the glutamine 74 residue with a glutamate residue (i.e., Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (i.e., a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., a Q74E mutation).

25 [0308] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 71. An example of such mutant IL-2 is the mutant 2-140.

SEQ ID NO: 71

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLTQLQSP

[0309] In one embodiment, the mutant IL-2 comprises at least one non-endogenous
 5 transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said
 motif:

- is inserted by insertion of a sequence comprising or consisting of QGA, at the C-terminus (*i.e.* comprises a sequence comprising or consisting of QGA at the C-terminus),

10 optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 substitution of the cysteine residue 125 with any other amino acid residue, preferably a
 serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with
 any other amino acid residue, preferably a glutamate residue (*i.e.*, Q74E mutation) or (iii)
 both a substitution of the cysteine residue 125 with any other amino acid residue,
 15 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 mutation).

[0310] In one embodiment, the sequence comprising or consisting of QGA is inserted at
 the C-terminus of the sequence with SEQ ID NO: 1.

20 [0311] In one embodiment, the sequence comprising or consisting of QGA is inserted at
 the C-terminus of the sequence with SEQ ID NO: 50.

[0312] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 61. An example of such mutant IL-2 is the mutant 2-126.

SEQ ID NO: 61

25 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLTQGA

[0313] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 188. An example of such mutant IL-2 is the mutant 2-629.

SEQ ID NO: 188

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKIFYMPKKATELK
 5 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLTQGA

[0314] In one embodiment, the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1.

10 [0315] Thus, in one embodiment, the mutant IL-2 comprises at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif:

- is inserted by insertion of a sequence comprising or consisting of QGA, at the C-terminus (*i.e.*, comprises a sequence comprising or consisting of QGA at the C-terminus), and

15 wherein the mutant IL-2 comprises a substitution of the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue (*i.e.*, F42A mutation), by reference to SEQ ID NO: 1,

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 20 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 25 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0316] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 72. An example of such mutant IL-2 is the mutant 2-141.

SEQ ID NO: 72

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLTQGA

[0317] The present invention further relates to a mutant IL-2 derived from the human
 5 IL-2 with SEQ ID NO: 1 or a variant thereof, comprising at least two non-endogenous
 transglutaminase motifs.

[0318] In one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID
 NO: 1 or a variant thereof further comprises a substitution of the cysteine residue 125
 with any other amino acid residue, preferably a serine residue (*i.e.* C125S mutation), by
 10 reference to SEQ ID NO: 1.

[0319] In one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID
 NO: 1 or a variant thereof further comprises a substitution of the glutamine 74 residue
 with any other amino acid residue, preferably a glutamate residue (*i.e.* Q74E mutation),
 by reference to SEQ ID NO: 1.

15 [0320] In one embodiment, the at least two non-endogenous transglutaminase motifs are
 as defined hereinabove.

[0321] In one embodiment, one of the non-endogenous transglutaminase motifs
 comprises the insertion of a Q residue at the T41 or F42 position as reference to SEQ ID
 NO: 1.

20 [0322] In one embodiment, the mutant IL-2 comprises at least two non-endogenous
 transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,
 wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID
 NO: 1 (*i.e.*, comprises a Q41 residue, preferably obtained by a T41Q mutation),
 25 and
- comprises or consists of the sequence LLQ, preferably comprises or consists of
 the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (*i.e.*, comprises a Q79 residue, preferably obtained by a H79Q mutation), and
- comprises or consists of the sequence LFQ, preferably comprises or consists of the sequence KLFQLRPRD (SEQ ID NO: 15),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0323] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 73. An example of such mutant IL-2 is the mutant 2-142.

SEQ ID NO: 73
APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKLFQLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

[0324] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation), and

- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence KLLQLRPRD (SEQ ID NO: 16),
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.* C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.* Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

10 [0325] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 74. An example of such mutant IL-2 is the mutant 2-143.

SEQ ID NO: 74

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKLLQLRPRDLISNINVIVLELKGSETTFMCEYAD
15 ETATIVEFLNRWITFSQSIISTLT

[0326] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- 25 - is inserted by insertion of a Q residue at the E100 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q100 residue, preferably obtained by a E100Q mutation), and
- comprises or consists of the sequence GSQ, preferably comprises or consists of the sequence GSQST (SEQ ID NO: 18),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0327] In one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID NO: 1 or a variant thereof comprises or consists of the sequence with SEQ ID NO: 75.
10 An example of such mutant IL-2 is the mutant 2-144.

SEQ ID NO: 75

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSQSTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

15 [0328] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation),
20 and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably
25 a sequence comprising or consisting of the sequence LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), at the N-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
30 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)

a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0329] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0330] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 76. An example of such mutant IL-2 is the mutant 2-145.

SEQ ID NO: 76

LQSPGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKK
ATELKHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFM
CEYADETATIVEFLNRWITFSQSIISTLT

[0331] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0332] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0333] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 77. An example of such mutant IL-2 is the mutant 2-146.

SEQ ID NO: 77

LQSPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATE
LKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

[0334] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
 - comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and
- wherein another motif:
- is inserted by insertion of a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of TQG,

preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus),

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

- [0335] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0336] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 78. An example of such mutant IL-2 is the mutant 2-147.

- SEQ ID NO: 78

TQGASSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

- [0337] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus

(*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus), optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0338] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSP (SEQ ID NO: 49), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0339] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 79. An example of such mutant IL-2 is the mutant 2-150.

SEQ ID NO: 79

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTLQSP

[0340] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of QGA, at the C-terminus (*i.e.* comprises a sequence comprising or consisting of QGA at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 5 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)
 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation),
 or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 10 mutation).

[0341] In one embodiment, the sequence comprising or consisting of QGA is inserted at
 the C-terminus of the sequence with SEQ ID NO: 1.

[0342] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 SEQ ID NO: 80. An example of such mutant IL-2 is the mutant 2-151.

15 SEQ ID NO: 80
 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLTQGA

[0343] In one embodiment, the mutant IL-2 comprises at least two non-endogenous
 20 transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,
 wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation),
 and
- 25 - comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably
 comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and
 wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation), and
- comprises or consists of the sequence LFQ, preferably comprises or consists of the sequence KLFQLRPRD (SEQ ID NO: 15),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0344] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 81. An example of such mutant IL-2 is the mutant 2-152.

SEQ ID NO: 81

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKLFQLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0345] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and
- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence KLLQLRPRD (SEQ ID NO: 16),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0346] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 82. An example of such mutant IL-2 is the mutant 2-153.

SEQ ID NO: 82
 APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTQRFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKLLQLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLT

[0347] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and
- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- is inserted by insertion of a Q residue at the E100 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q100 residue, preferably obtained by a E100Q mutation), and

- comprises or consists of the sequence GSQ, preferably comprises or consists of the sequence GSQST (SEQ ID NO: 18),
optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)
5 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation),
or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

10 [0348] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 83. An example of such mutant IL-2 is the mutant 2-154.

SEQ ID NO: 83

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVNLNLAESKNFHLRPRDLISNINVIVLELKGSQSTFMCEYA
15 DETATIVEFLNRWITFSQSIISTLT

[0349] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,
wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID
20 NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation),
and
- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably
comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- 25 - is inserted by insertion of a sequence comprising or consisting of LQS, preferably
a sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO:
40), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS,
preferably a sequence comprising or consisting of LQSPGAPTSSSTKKTQ, at the
N-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0350] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0351] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 84. An example of such mutant IL-2 is the mutant 2-155.

SEQ ID NO: 84

LQSPGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKK
ATELKHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFM
CEYADETATIVEFLNRWITFSQSIISTLT

[0352] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and
 - comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and
- wherein another motif:
- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS,

preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus),

optionally wherein the mutant IL-2 comprises a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0353] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0354] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 85. An example of such mutant IL-2 is the mutant 2-156.

SEQ ID NO: 85

15 LQSPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATE
LKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFSQSIISTLT

[0355] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and
- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of TQG,

preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus),

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

- [0356] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0357] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 86. An example of such mutant IL-2 is the mutant 2-157.

- SEQ ID NO: 86
TQGASSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

- [0358] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and
- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-

terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
 5 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)
 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation),
 or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue,
 preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
 10 mutation).

[0359] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0360] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
 15 SEQ ID NO: 87. An example of such mutant IL-2 is the mutant 2-158.

SEQ ID NO: 87

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
 KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
 DETATIVEFLNRWITFSQSIISTLTGGLQSP

20 [0361] In one embodiment, the mutant IL-2 comprises at least two non-endogenous
 transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,
 wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID
 NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation),
 25 and

- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably
 comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48) at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48), at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0362] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GLQSP (SEQ ID NO: 48), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0363] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 88. An example of such mutant IL-2 is the mutant 2-159.

SEQ ID NO: 88

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLTGLQSP

[0364] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and
- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting LQSP (SEQ ID NO: 49), at the C-terminus),
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0365] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSP (SEQ ID NO: 49), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0366] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 89. An example of such mutant IL-2 is the mutant 2-160.

SEQ ID NO: 89

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLTLQSP

[0367] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the F42 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q42 residue, preferably obtained by a F42Q mutation), and
- comprises or consists of the sequence LTQR (SEQ ID NO: 2), preferably comprises or consists of the sequence MLTQRF (SEQ ID NO: 12), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of QGA, at the C-terminus (*i.e.* comprises a sequence comprising or consisting of QGA at the C-terminus),

- 5 optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74
- 10 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0368] In one embodiment, the sequence comprising or consisting of QGA is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

- [0369] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
- 15 SEQ ID NO: 90. An example of such mutant IL-2 is the mutant 2-161.

SEQ ID NO: 90

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTQRFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLTQGA

- 20 [0370] In one embodiment, the mutant IL-2 comprises at least three non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,

wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation),

- 25 and

- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a Q residue at the T51 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q51 residue, preferably obtained by a T51Q mutation), and
- comprises or consists of the sequence AQA, preferably comprises or consists of the sequence LAQALK (SEQ ID NO: 106), and

wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence KLLQLRPRD (SEQ ID NO: 16),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0371] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 119. An example of such mutant IL-2 is the mutant 2-127.

SEQ ID NO: 119

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKLAQAL
KHLQCLEEELKPLEEVLNLAESKLLQLRPRDLISNINVIVLELKGSETTFMCEYA
DETATIVEFLNRWITFSQSIISTLT

[0372] In one embodiment, the mutant IL-2 comprises at least three non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and

- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (i.e. comprises a Q79 residue, preferably obtained by a H79Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence KLLQLRPRD (SEQ ID NO: 16), and

wherein another motif:

- is inserted by insertion of a Q residue at the E110 position by reference to SEQ ID NO: 1 (i.e. comprises a Q110 residue, preferably obtained by a E110Q mutation), and
- comprises or consists of the sequence ALQ, preferably comprises or consists of the sequence DYALQ (SEQ ID NO: 109)

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0373] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 120. An example of such mutant IL-2 is the mutant 2-128.

SEQ ID NO: 120

- APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKLLQLRPRDLISNINVIVLELKGSETTFMCDYAL
QTATIVEFLNRWITFSQSIISTLT

[0374] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,

- wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0375] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 123. An example of such mutant IL-2 is the mutant 2-148.

SEQ ID NO: 123

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTGGLQSP

[0376] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and

- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQAKF (SEQ ID NO: 11), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48) at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48), at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0377] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GLQSP (SEQ ID NO: 48), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0378] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 124. An example of such mutant IL-2 is the mutant 2-149.

SEQ ID NO: 124

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQAKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTGLQSP

[0379] In one embodiment, the mutant IL-2 derived from the human IL-2 with SEQ ID NO: 1 or a variant thereof, comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10).

[0380] In one embodiment, the other non-endogenous transglutaminase motif is as defined hereinabove.

[0381] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,

10 wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

15 wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation), and
- comprises or consists of the sequence LFQ, preferably comprises or consists of the sequence KLFQLRPRD (SEQ ID NO: 15),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation),
25 or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0382] In one embodiment, the mutant IL-2 comprises or consists of the sequence with
30 SEQ ID NO: 91. An example of such mutant IL-2 is the mutant 2-162.

SEQ ID NO: 91

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRLLQFKFYMPKKATELK
HLQCLEELKPLEEVNLNLAESKLFQLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLT

- 5 [0383] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation),
- 10 and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

wherein another motif:

- is inserted by insertion of a Q residue at the H79 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q79 residue, preferably obtained by a H79Q mutation),
- 15 and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence KLLQLRPRD (SEQ ID NO: 16),

- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a
- 20 substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E
- 25 mutation).

[0384] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 92. An example of such mutant IL-2 is the mutant 2-163.

SEQ ID NO: 92

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKLLQLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLT

[0385] In one embodiment, the mutant IL-2 comprises at least two non-endogenous
 5 transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,
 wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- 10 - comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

wherein another motif:

- is inserted by insertion of a Q residue at the E100 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q100 residue, preferably obtained by a E100Q mutation), and
- 15 - comprises or consists of the sequence GSQ, preferably comprises or consists of the sequence GSQST (SEQ ID NO: 18),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii)
 20 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

25 [0386] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 93. An example of such mutant IL-2 is the mutant 2-164.

SEQ ID NO: 93

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSTTFMCEYAD
 30 ETATIVEFLNRWITFSQSIISTLT

[0387] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), at the N-terminus),
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0388] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPTSSSTKKTQ (SEQ ID NO: 42), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

- [0389] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 94. An example of such mutant IL-2 is the mutant 2-166.

SEQ ID NO: 94

LQSPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATE
LKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEY

- ADETATIVEFLNRWITFSQSIISTLT

[0390] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of TQG, preferably a sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), at the N-terminus),
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0391] In one embodiment, the sequence comprising or consisting of TQG, preferably the sequence comprising or consisting of TQGASSTKKTQ (SEQ ID NO: 44), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

- [0392] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 95. An example of such mutant IL-2 is the mutant 2-167.

SEQ ID NO: 95

TQGASSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRLLQFKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYA

- DETATIVEFLNRWITFSQSIISTLT

[0393] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSP (SEQ ID NO: 49), at the C-terminus), optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0394] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSP (SEQ ID NO: 49), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0395] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 96. An example of such mutant IL-2 is the mutant 2-170.

SEQ ID NO: 96

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTLQSP

[0396] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of QGA, at the C-terminus (*i.e.* comprises a sequence comprising or consisting of QGA at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0397] In one embodiment, the sequence comprising or consisting of QGA is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0398] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 97. An example of such mutant IL-2 is the mutant 2-171.

SEQ ID NO: 97

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFSQSIISTLTQGA

[0399] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,

wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- 5 - comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence LLQFKF (SEQ ID NO: 10), and

wherein another motif:

- is inserted by insertion of a Q residue at the T51 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q51 residue, preferably obtained by a T51Q mutation), and
- 10 - comprises or consists of the sequence AQA, preferably comprises or consists of the sequence LAQALK (SEQ ID NO: 106)

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) 15 a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

20 [0400] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 121. An example of such mutant IL-2 is the mutant 2-129.

SEQ ID NO: 121

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKLAQALK
HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
25 ETATIVEFLNRWITFSQSIISTLT

[0401] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence preferably LLQFKF (SEQ ID NO: 10),

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of the sequence LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), at the N-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), at the N-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0402] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), is inserted at the N-terminus of the sequence with SEQ ID NO: 50.

[0403] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 125. An example of such mutant IL-2 is the mutant 2-165.

SEQ ID NO: 125

25 LQSPGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKK
ATELKHLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFM
CEYADETATIVEFLNRWITFSQSIISTLT

[0404] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof,

wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- 5 - comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence preferably LLQFKF (SEQ ID NO: 10),

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), at the C-terminus),

optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0405] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GGLQSP (SEQ ID NO: 47), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

[0406] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 126. An example of such mutant IL-2 is the mutant 2-168.

25 SEQ ID NO: 126
 APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
 HLQCLEEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
 ETATIVEFLNRWITFSQSIISTLTGGLQSP

[0407] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs as compared to SEQ ID NO: 1 or variant thereof, wherein one motif:

- is inserted by insertion of a Q residue at the T41 position by reference to SEQ ID NO: 1 (*i.e.* comprises a Q41 residue, preferably obtained by a T41Q mutation), and
- comprises or consists of the sequence LLQ, preferably comprises or consists of the sequence preferably LLQFKF (SEQ ID NO: 10),

wherein another motif:

- is inserted by insertion of a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48) at the C-terminus (*i.e.* comprises a sequence comprising or consisting of LQS, preferably a sequence comprising or consisting of GLQSP (SEQ ID NO: 48), at the C-terminus),
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with a serine residue (*i.e.*, C125S mutation), (ii) a substitution of the glutamine 74 residue with a glutamate residue (*i.e.*, Q74E mutation), or (iii) both a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).

[0408] In one embodiment, the sequence comprising or consisting of LQS, preferably the sequence comprising or consisting of GLQSP (SEQ ID NO: 48), is inserted at the C-terminus of the sequence with SEQ ID NO: 1.

- [0409] In one embodiment, the mutant IL-2 comprises or consists of the sequence with SEQ ID NO: 127. An example of such mutant IL-2 is the mutant 2-169.

SEQ ID NO: 127

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRLLQFKFYMPKKATELK
HLQCLEELKPLEEVLNLAESKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD

- ETATIVEFLNRWITFSQSIISTLTGLQSP

[0410] The present invention further relates to a method for incorporating at least one non-endogenous transglutaminase motif in human IL-2 with the sequence SEQ ID NO: 1 or from a variant thereof, comprising the steps of:

- inserting a Q residue at one or more of the following positions by reference to
5 SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, Q57, H79, E95, E100, E110 and S130, and/or
- inserting a motif at the N-terminus or C-terminus, wherein said motif comprises a sequence selected from the group comprising or consisting of LQS and TQG, optionally wherein said method further comprises the steps of:
10 (i) substituting the cysteine residue 125 with any other amino acid residue, preferably a serine residue (i.e., a C125S mutation),
(ii) substituting the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., a Q74E mutation), or
(iii) both substituting the cysteine residue 125 with any other amino acid residue,
15 preferably a serine residue (i.e., a C125S mutation) and substituting the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (i.e., a Q74E mutation).

[0411] In one embodiment, the method further comprises a step of substituting the phenylalanine 42 residue with any other amino acid residue, preferably an alanine residue
20 (i.e., F42A mutation), by reference to SEQ ID NO: 1.

[0412] In one embodiment, the method comprises the insertion of at least two non-endogenous transglutaminase motifs.

[0413] In one embodiment, one of the non-endogenous transglutaminase motifs comprises the insertion of a Q residue at the T41 or F42 position as reference to SEQ ID
25 NO: 1.

[0414] The embodiments described herein for the mutant IL-2 apply *mutatis mutandis* to the method as described herein.

[0415] The present invention further relates to a fusion protein comprising a mutant IL-2 as defined hereinabove.

[0416] In one embodiment, the fusion protein comprises at least one masking moiety, wherein the at least one masking moiety is linked to a Q residue of the at least one non-endogenous transglutaminase motif of the mutant IL-2 as defined hereinabove.

5 [0417] In one embodiment, the mutant IL-2 comprises at least two non-endogenous transglutaminase motifs, and the fusion protein comprises at least two masking moieties, wherein the at least two masking moieties are linked to a Q residue of each non-endogenous transglutaminase motif of the mutant IL-2 as defined hereinabove.

[0418] In one embodiment, when the fusion protein comprises at least two masking moieties, the at least two masking moieties are different. In one embodiment, when the
10 fusion protein comprises at least two masking moieties, the at least two masking moieties are similar.

[0419] As used herein, the masking moiety refers to a moiety that, when linked to a Q residue of the non-endogenous transglutaminase motif of the mutant IL-2, prevents or reduces the signaling of the mutant IL-2, or the binding or affinity of the mutant IL-2 to
15 its receptor.

[0420] Methods for measuring the IL-2 signaling are well known in the art and comprise, for example, the measurement of the induction of IL-2 receptor signaling (e.g., by detection of phosphorylated STAT5a), the measurement of the induction of T cell proliferation (e.g., by detection of Ki-67 using in particular CellTrace™ Cell Proliferation
20 Kits, by direct assessment of T cell proliferation in the presence of IL-2, in T cell activation assay experiments (comprising, for example, the activation of cells with CD3 and CD28 in the presence of IL-2), or using cell lines that depend on IL-2 to proliferate, such as, for example CTLL2 cell line) and/or the measurement of an up-regulation of expression of activation markers (such as e.g., CD25, CD69, cytotoxic molecules, such
25 as, for example, granzyme B, and the like).

[0421] Affinity of a protein is commonly expressed as an equilibrium dissociation constant (K_D). Thus, in one embodiment, the binding kinetics, such as the K_D , of the at mutant IL-2 to its receptor are determined by surface plasmon resonance (SPR, e.g., using BIAcore®).

[0422] Other methods for measuring binding or affinity of proteins are well known in the art, and include, for example, cell-binding assays, such as competitive binding assays.

[0423] In one embodiment, the mutant IL-2 comprised in the fusion protein as defined hereinabove has a reduced binding or affinity for its receptor as compared to the mutant IL-2 that is free (*i.e.*, not associated with a masking moiety). In one embodiment, the mutant IL-2 comprised in the fusion protein as defined hereinabove has a reduced binding or affinity for its receptor by at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95% or more as compared to the mutant IL-2 that is free (*i.e.*, not associated with a masking moiety).

[0424] In one embodiment, the mutant IL-2 comprised in the fusion protein as defined hereinabove has a reduced binding or affinity for the IL-2R alpha, beta and/or gamma chain as compared to the mutant IL-2 that is free (*i.e.*, not associated with a masking moiety). In one embodiment, the mutant IL-2 comprised in the fusion protein as defined hereinabove has a reduced binding or affinity for the IL-2R alpha, beta and/or gamma chain by at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95% or more as compared to the mutant IL-2 that is free (*i.e.*, not associated with a masking moiety).

[0425] In one embodiment, the mutant IL-2 comprised in the fusion protein as defined hereinabove has a reduced binding or affinity for the low-affinity, intermediate-affinity, and/or high-affinity IL-2 receptor as compared to the mutant IL-2 that is free (*i.e.*, not associated with a masking moiety). In one embodiment, the mutant IL-2 comprised in the fusion protein as defined hereinabove has a reduced binding or affinity for the low-affinity, intermediate-affinity, and/or high-affinity IL-2 receptor by at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95% or more as compared to the mutant IL-2 that is free (*i.e.*, not associated with a masking moiety).

[0426] As disclosed herein, the at least one masking moiety may be any type of molecule.

[0427] In one embodiment, the at least one masking moiety is selected from the group comprising or consisting of glycans, lipids, proteins, peptides, small organic molecule and polymers.

[0428] In one embodiment, the at least one masking moiety is a glycan.

- 5 [0429] In one embodiment, the at least one masking moiety is a polymer, preferably a water-soluble polymer.

[0430] In one embodiment, the water-soluble polymer is selected from the group comprising or consisting of polyethylene glycol (PEG), poly(propylene glycol) (PPG), copolymers of ethylene glycol and propylene glycol, poly(oxyethylated polyol),
10 poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), and combinations thereof.

[0431] In one embodiment, the water-soluble polymer is a polyethylene glycol (PEG).

- 15 [0432] In one embodiment, the at least one masking moiety is a linear PEG. As used herein, a linear PEG refers to a PEG composed of one linear chain.

[0433] In one embodiment, the at least one masking moiety is a linear PEG up to about 20kDa, preferably of about 10 kDa.

- 20 [0434] In one embodiment, the at least one masking moiety is a branched PEG. As used herein, a branched PEG refers to a PEG composed of at least two linear PEGs that are linked together. An example of a branched PEG is a Y-shaped PEGs, such as, for example, a Y-shaped PEG with two PEGs of about 20 kDa.

[0435] In one embodiment, the at least one masking moiety is a PEG having a weight comprised from about 5 to about 50 kDa, preferably from about 10 to about 40 kDa.

- 25 [0436] It will be understood that, when the fusion protein comprises at least two masking moieties, the at least two masking moieties may be PEG that are similar or different.

[0437] In one embodiment, the fusion protein further comprises at least one binding moiety, such as, for example, antibodies or fragments thereof, or antibody mimetics. As used herein, a binding moiety refers to any molecule capable of binding to a target.

[0438] In one embodiment, the binding moiety and the masking moiety are one single molecule, *i.e.* the single molecule is able to act as both a masking moiety and a binding moiety.

[0439] In one embodiment, the fusion protein further comprises at least one antibody or antigen-binding fragment thereof.

[0440] In one embodiment, the at least one antibody or antigen-binding fragment thereof comprised in the fusion protein is selected from the group comprising or consisting of a whole antibody, a single chain antibody, a dimeric single chain antibody, a Fv, a Fab, a Fab', a Fab'-SH, a F(ab')₂, a Fd, a defucosylated antibody, a bispecific antibody, a diabody, a triabody, a tetrabody, a nanobody, a domain antibody, and a unibody, preferably the at least one antibody or antigen-binding fragment is a Fab.

[0441] In one embodiment, the at least one antibody or antigen-binding fragment thereof is polyclonal. In one embodiment, the at least one antibody or antigen-binding fragment thereof is monoclonal.

[0442] In one embodiment, the at least one antibody or antigen-binding fragment thereof is humanized. In one embodiment, the at least one antibody or antigen-binding fragment thereof comprised in the fusion protein is a fully or substantially human antibody or fragment thereof.

[0443] It will be appreciated that antibodies or antigen-binding fragments thereof comprised in a fusion protein according to the present invention can be modified using known methods.

[0444] Modifications can also have been made to framework residues within the heavy chain variable region (VH) and/or the light chain variable region (VL), *e.g.*, to improve the properties of the antibody or fragment. Typically, such framework modifications are made to decrease the immunogenicity of the antibody or fragment. For example, one

approach is to “back-mutate” one or more framework residues to the corresponding germline sequence.

[0445] As another example, antibodies or antigen-binding fragments thereof can be aglycosylated (*i.e.*, the antibody or fragment lacks glycosylation). Glycosylation can be
5 altered to, for example, increase the affinity of the antibody or fragment for the antigen or alter the antibody-dependent cellular cytotoxicity activity of the antibody or fragment thereof. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence.

[0446] In another embodiment, the fusion protein further comprises at least one antibody
10 mimetic. Examples of antibody mimetic include, without limitation, affibody, an affilin, an affitin, an adnectin, an atrimer, an evasin, a DARPin, an anticalin, an avimer, a fynomer, and a versabody

[0447] In one embodiment, the at least one antibody or antigen-binding fragment or the
at least one antibody mimetic targets an antigen, such as, for example, a cancer antigen
15 or an immune checkpoint protein.

[0448] Examples of cancer antigens include, without limitation, EGFR, HER2, HER3, HER 4, NY-ESO -1, GPC -3, CLL -1, BCMA, GD2, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CCL27, CCL28, CX3CR1, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6), Mucin family (MUC1, MUC2 MUC3 A, MUC3 B, MUC4, MUC5 AC, MUC5
20 B, MUC6, MUC7, MUC8, MUC12, MUC6, MUCUNA, MUC16, MUC17, MUCUNA, MUCIN, TERT, TLR, Survivin, CD123, TIGIT, DLL3, PSMA, Fibroblast Growth Factor Receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), hepatocyte growth factor receptor (HGFR), nerve growth factor receptor (NGFR), insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR) or
25 hormone receptor (melanocortin 1 receptor (MC 1R, MSHR).

[0449] Examples of immune checkpoint proteins include, without limitation, PD-1, PD-L1, CTLA-4, LAG-3, OX40, CD28, CD40, CD47, CD70, CD80, CD122, GTIR, A2AR, B7-H3 (CD276), B7-H4, IDO, KIR, TIM -3 or 4-1 BB (CD137).

[0450] In one embodiment, the at least one antibody or antigen-binding fragment or antibody mimetic targets HER2. As used herein, HER2 (human epidermal growth factor receptor 2, also known as CD340 (cluster of differentiation 340)) is a protein encoded by the *ERBB2* gene.

- 5 [0451] In one embodiment, the at least one antibody or antigen-binding fragment or antibody mimetic targets PD-1. As used herein, PD-1 (Programmed cell death protein 1, also known as CD279 (cluster of differentiation 279)) is a protein encoded by *PDCD1* gene.

- 10 [0452] In one embodiment, the fusion protein comprises at least one mutant IL-2, at least one masking moiety and at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, wherein the at least one masking moiety is linked to the at least one mutant IL-2 with a linker and wherein the at least one mutant IL-2 is linked to the at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, with another linker.

- 15 [0453] In one embodiment, the linkers linking (i) the at least one masking moiety to the at least one mutant IL-2 and (ii) the at least one mutant IL-2 to the at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, are similar. In one embodiment, the linkers linking (i) the at least one masking moiety to the at least one mutant IL-2 and (ii) the at least one mutant IL-2 to the at least
20 one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, are different.

- [0454] In one embodiment, the fusion protein comprises at least one mutant IL-2, at least two masking moieties and at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, wherein the at least two masking
25 moieties are linked to the at least one mutant IL-2 with a linker.

[0455] In one embodiment, the linkers linking the at least two masking moieties to the at least one mutant IL-2 are similar. In one embodiment, the linkers linking the at least two masking moieties to the at least one mutant IL-2 are different.

[0456] In one embodiment, the linker linking the at least one mutant IL-2 to the at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, is a cleavable linker. In another embodiment, the linker linking the at least one mutant IL-2 to the at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, is an uncleavable linker. It will be understood that the nature of the linker (*i.e.*, cleavable or uncleavable) will depend on the therapeutic applications, since, depending on the conditions, it may be desirable to have either a releasable IL-2 or an IL-2 irreversibly linked to the binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic.

10 [0457] In one embodiment, the linker linking the at least one masking moiety to the at least one mutant IL-2 is a cleavable linker. In another embodiment, the linker linking the at least one masking moiety to the at least one mutant IL-2 is an uncleavable linker. It will be understood that the nature of the linker (*i.e.*, cleavable or uncleavable) will depend on the therapeutic applications, since, depending on the conditions, it may be desirable to have either a releasable masking moiety or an IL-2 irreversibly linked to the masking moiety.

[0458] In one embodiment, the fusion protein comprises at least one or at least two masking moiety(ies), at least one mutant IL-2 and at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, wherein said elements are linked by cleavable linkers.

[0459] In one embodiment, the fusion protein comprises at least one or at least two masking moiety(ies), at least one mutant IL-2 and at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, wherein said elements are linked by uncleavable linkers.

25 [0460] In one embodiment, the fusion protein comprises at least one or at least two masking moiety(ies), at least one mutant IL-2 and at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, wherein the at least one or at least two masking moiety(ies) are linked to the at least one mutant IL-2 with an uncleavable linker, and wherein the at least one binding moiety, preferably an

antibody or antigen-binding fragment thereof or an antibody mimetic, is linked to the at least one mutant IL-2 with a cleavable linker.

[0461] In one embodiment, the fusion protein comprises at least one or at least two masking moiety(ies), at least one mutant IL-2 and at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, wherein the at least one or at least two masking moiety(ies) are linked to the at least one mutant IL-2 with a cleavable linker, and wherein the at least one binding moiety, preferably an antibody or antigen-binding fragment thereof or an antibody mimetic, is linked to the at least one mutant IL-2 with an uncleavable linker.

10 [0462] In one embodiment, the uncleavable linker is a peptide having a length ranging from 2 and 10 amino acids.

[0463] For example, a glycine-serine doublet provides a particularly suitable hinge domain (GS linker). In one embodiment, the hinge domain is a Gly/Ser linker. Examples of Gly/Ser linkers include, but are not limited to, GS linkers, G₂S linkers, G₃S linkers, 15 G₄S linkers.

[0464] Examples of G₂S linkers include, but are not limited to, GGS.

[0465] G₃S linkers comprise the amino acid sequence (Gly-Gly-Gly-Ser)_n also referred to as (GGGS)_n (SEQ ID NO: 100), where n is a positive integer equal to or greater than 1 (such as, example, n=1, n=2, n=3, n=4, n=5, n=6, n=7, n=8, n=9 or n=10).

20 [0466] G₄S linkers comprise the amino acid sequence (Gly-Gly-Gly-Gly-Ser)_n also referred to as (GGGGS)_n (SEQ ID NO: 101), where n is a positive integer equal to or greater than 1 (such as, example, n=1, n=2, n=3, n=4, n=5, n=6, n=7, n=8, n=9 or n=10).

[0467] As another example, linkers A₄T (SEQ ID NO: 129) may be also used in the context of the present invention.

25 [0468] In one embodiment, the cleavable linker is a cleavable peptide having a length ranging from 2 and 10 amino acids.

[0469] In one embodiment, the cleavable linker is cleavable by enzymes, photo-irradiation, pH, or chemical agents.

[0470] In one embodiment, the cleavable linker is cleavable by enzymes, such as, for example, linkers cleavable by proteases, linkers cleavable by beta glucuronidase or
5 peptide linkers.

[0471] In one embodiment, the cleavable linker is cleavable by pH. Examples of linker cleavable by pH include, without limitation, hydrazine linker, maleic acid amide linker or cis-aconityl linker.

[0472] An example of a fusion protein of the present invention is described in **Figure**
10 **14**. The fusion protein of the present invention may or may not have a binding moiety, and may or may not have a linker between the binding moiety and the IL-2 mutant. Regarding the linkers, the linker between the binding moiety and the IL-2 mutant may or may not be proteolytically cleavable, and the linker between the masking moiety and the IL-2 may or may not be cleavable.

15 [0473] Another object of the invention is an isolated nucleic acid encoding a mutant IL-2 or a fusion protein according to the present invention.

[0474] An “**isolated nucleic acid**”, as used herein, is intended to refer to a nucleic acid that is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native
20 sequence. The term embraces a nucleic acid sequence that has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure nucleic acid includes isolated forms of the nucleic acid. Of course, this refers to the nucleic acid as originally isolated and does not exclude genes
25 or sequences later added to the isolated nucleic acid by the hand of man.

[0475] Typically, said nucleic acid is a DNA or RNA molecule, which may be included in any suitable vector, such as for example plasmid, cosmid, episome, artificial chromosome, phage or a viral vector.

[0476] Thus, another object of the present invention is an expression vector comprising a nucleic acid encoding a mutant IL-2 or a fusion protein according to the present invention.

[0477] The terms “**vector**”, “**cloning vector**” and “**expression vector**” mean the vehicle
5 by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform a host and promote expression (e.g. transcription and translation) of the introduced sequence. Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said fusion protein upon administration to a host. Examples of promoters and enhancers used in the
10 expression vector for animal cell include early promoter and enhancer of SV40, LTR promoter and enhancer of Moloney mouse leukemia virus, promoter and enhancer of immunoglobulin H chain and the like. Any expression vector for animal cell can be used, so long as a gene encoding the fusion protein can be inserted and expressed. Examples of suitable vectors include pAGE107, pAGE103, pHSG274, pKCR, pSG1 beta d2-4 and the
15 like. Other examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Other examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or
20 viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, Gpenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found in the art.

[0478] Another object of the invention is an isolated host cell comprising said vector. Said host cell may be used for the recombinant production of a mutant IL-2 or a fusion
25 protein according to the present invention.

[0479] In an embodiment, host cells may be prokaryote, yeast, or eukaryote cells, preferably mammalian cells, such as, for example: monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen. Virol. 36:59 (1977));
30 baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary

cells/-DHFR (CHO, Urlaub *et al.*, Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse Sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); mouse myeloma cells SP2/0-AG14 (ATCC CRL 1581 ; ATCC CRL 8287) or NSO (HPA culture collections no. 85110503); monkey kidney cells (CV1 ATCC CCL 70); African green
5 monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci. 383:44-68 (1982));
10 MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2), as well as DSM's PERC-6 cell line. Expression vectors suitable for use in each of these host cells are also generally known in the art. It should be noted that the term “**host cell**” generally refers to a cultured cell line. In one embodiment, whole human beings into which an expression vector encoding a fusion protein according to the invention has been
15 introduced are excluded from the definition of a “**host cell**”.

[0480] Another object of the present invention is a composition comprising, consisting essentially of or consisting of at least one mutant IL-2, at least one fusion protein, at least one nucleic acid, or a at least one expression vector according to the present invention.

[0481] As used herein, “**consisting essentially of**”, with reference to a composition,
20 means that the at least one mutant IL-2, fusion protein, nucleic acid or expression vector is the only one therapeutic agent or agent with a biologic activity within said composition.

[0482] Another object of the present invention is a pharmaceutical composition comprising, consisting essentially of or consisting of at least one mutant IL-2, at least one fusion protein, at least one nucleic acid, or a at least one expression vector according to
25 the present invention, and at least one pharmaceutically acceptable excipient.

[0483] The term “pharmaceutically acceptable excipient” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. Said excipient does not produce an adverse, allergic or other untoward reaction when administered to an animal, preferably a mammal, more

preferably a human. For human administration, preparations should meet sterility, pyrogenicity, and general safety and purity standards as required by regulatory offices, such as, for example, FDA Office or EMA.

[0484] Examples of pharmaceutically acceptable excipients that may be used in the compositions of the present invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances (for example sodium carboxymethylcellulose), polyethylene glycol, polyacrylates, waxes, polyethylene- polyoxypropylene- block polymers, polyethylene glycol and wool fat.

[0485] In one embodiment, the pharmaceutical compositions according to the present invention comprise vehicles which are pharmaceutically acceptable for a formulation capable of being injected to a subject. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

[0486] Another object of the present invention is a mutant IL-2, a fusion protein, a nucleic acid, or an expression vector according to the present invention for use as a medicament.

[0487] Another object of the present invention is a medicament comprising, consisting essentially of or consisting of at least one mutant IL-2, at least one fusion protein, at least one nucleic acid, or at least one expression vector according to the present invention.

[0488] Another object of the present invention is the use of at least one mutant IL-2, at least one fusion protein, at least one nucleic acid, or at least one expression vector in the

manufacture of a medicament for treating a disease, disorder or symptoms in a subject in need thereof.

[0489] For use in administration to a subject, the composition, pharmaceutical composition or medicament will be formulated for administration to the subject.

- 5 [0490] In one embodiment, the composition, pharmaceutical composition or medicament according to the present invention is administered (or is to be administered) parenterally, by inhalation spray, rectally, nasally, or via an implanted reservoir.

- [0491] In one embodiment, the composition, pharmaceutical composition or medicament is administered (or is to be administered) by injection, including, without
10 limitation, subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection, or infusion techniques.

- [0492] Examples of forms adapted for injection include, but are not limited to, solutions, such as, for example, sterile aqueous solutions, gels, dispersions, emulsions, suspensions,
15 solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to use, such as, for example, powder, liposomal forms, and the like.

- [0493] Sterile injectable forms of the compositions, pharmaceutical compositions or medicaments of this invention may be aqueous or an oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable
20 dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For
25 this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as

carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0494] In one embodiment, the mutant IL-2, fusion protein, nucleic acid, expression vector, composition, pharmaceutical composition or medicament according to the present invention is to be administered to the subject in need thereof in a therapeutically effective amount.

[0495] It will be however understood that the total daily usage of the IL-2 mutant, fusion protein, nucleic acid, expression vector, composition, pharmaceutical composition or medicament according to the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disease being treated and the severity of the disease; activity of the IL-2 mutant, fusion protein, nucleic acid, or expression vector employed; the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific IL-2 mutant, fusion protein, nucleic acid, or expression vector employed; the duration of the treatment; drugs used in combination or coincidental with the specific IL-2 mutant, fusion protein, nucleic acid, or expression vector employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. The total dose required for each treatment may be administered by multiple doses or in a single dose.

[0496] The present invention also relates to at least one mutant IL-2, fusion protein, nucleic acid or expression vector as described herein for use as a medicament, *i.e.*, for treating (or for use in treating) diseases, disorders or symptoms in a subject in need thereof.

[0497] The present invention also relates to at least one mutant IL-2, fusion protein, nucleic acid or expression vector as described herein for use in promoting Treg cell expansion in a subject in need thereof, thereby treating diseases, disorders or symptoms in the subject.

- 5 [0498] The present invention also relates to methods for treating diseases, disorders or symptoms or methods for promoting Treg cell expansion in a subject in need thereof, comprising administering to the subject the at least one mutant IL-2, fusion protein, nucleic acid or expression vector as described herein.

[0499] Examples of diseases that may be treated in the present invention, include, but
10 are not limited to, cancers, and inflammatory diseases.

[0500] In one embodiment, the disease, disorder or symptom to be treated is cancer.

- [0501] As used herein, the term “**cancer**” has its general meaning in the art and includes, but is not limited to, solid tumors and blood borne tumors. The term cancer includes, without limitation, diseases of the skin, tissues, organs, bone, cartilage, blood and vessels.
15 The term “cancer” further encompasses both primary and metastatic cancers.

[0502] Examples of cancers that may treated by methods and compositions of the invention include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, endometrial, pancreas
20 or uterus.

- [0503] In addition, the cancer may be selected in the following non-limiting list: malignant neoplasm; undifferentiated carcinoma; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma;
25 papillary transitional cell carcinoma; adenocarcinoma; malignant gastrinoma; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma associated with familial

polyposis coli; solid carcinoma; malignant carcinoid tumor; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular
5 adenocarcinoma; non-encapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet
10 ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease of the breast; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma with squamous metaplasia; malignant thymoma; malignant ovarian stromal tumor; malignant thecoma; malignant granulosa cell tumor; malignant roblastoma; Sertoli cell carcinoma; malignant leydig cell tumor;
15 malignant lipid cell tumor; malignant paraganglioma; malignant extra-mammary paraganglioma; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malign melanoma in giant pigmented nevus; epithelioid cell melanoma; malignant blue nevus; sarcoma; fibrosarcoma; malignant fibrous histiocytoma; myxosarcoma; liposarcoma;
20 leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; malignant mixed tumor; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; malignant mesenchymoma; malignant brenner tumor; malignant phyllodes tumor; synovial sarcoma; malignant mesothelioma; dysgerminoma; embryonal carcinoma; malignant teratoma; malignant
25 struma ovarii; choriocarcinoma; malignant mesonephroma; hemangiosarcoma; malignant hemangioendothelioma; kaposi's sarcoma; malignant hemangiopericytoma; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; malignant chondroblastoma; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; malignant odontogenic tumor; ameloblastic odontosarcoma; malignant
30 ameloblastoma; ameloblastic fibrosarcoma; malignant pinealoma; chordoma; malignant glioma; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive

neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; malignant meningioma; neurofibrosarcoma; malignant neurilemmoma; malignant granular cell tumor; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma - small
 5 lymphocytic; malignant diffuse large cell lymphoma; malignant follicular lymphoma; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic
 10 leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

[0504] In one embodiment, the disease, disorder or symptom to be treated is an inflammatory disease.

[0505] Examples of inflammatory diseases include, but are not limited to, arthritis,
 15 rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, psoriatic arthritis, juvenile idiopathic arthritis, juvenile rheumatoid arthritis, arthritis uratica, gout, chronic polyarthritis, periartthritis humeroscapularis, cervical arthritis, lumbosacral arthritis, enteropathic arthritis and ankylosing spondylitis, asthma, dermatitis, psoriasis, scleroderma, polymyositis, dermatomyositis, juvenila dermatomyositis, primary biliary
 20 cirrhosis, fibrosis, cystic fibrosis, pulmonary fibrosis, cirrhosis, endomyocardial fibrosis, dediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, nephrogenic fibrosis, Keloids, scleroderma, arthrofibrosis, post transplantation late and chronic solid organ rejection, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, pemphigus, Pemphigus vulgaris, Pemphigus herpetiformis, Pemphigus vegetans, IgA pemphigus, Pemphigus
 25 erythematosus, bullous pemphigoid, Pemphigoid gestationis, Mucous membrane dermatosis, Pemphigoid nodularis, Linear IgA bullous dermatosis, Bullous lichen planus, Epidermolysis bullosa acquisita, autoimmune diabetes, diabetic retinopathy, diabetic nephropathy, diabetic vasculopathy, ocular inflammation, uveitis, rhinitis, ischemia-reperfusion injury, post-angioplasty restenosis, chronic obstructive pulmonary disease
 30 (COPD), glomerulonephritis, Graves disease, gastrointestinal allergies, conjunctivitis,

atherosclerosis, coronary artery disease, angina, small artery disease, acute disseminated encephalomyelitis, idiopathic thrombocytopenic purpura, multiple sclerosis, systemic sclerosis, antiphospholipid syndrome, Sjogren's syndrome, autoimmune hemolytic anemia, colitis, Crohn's Disease, ulcerative colitis, Inflammatory Bowel Disease (IBD),
5 embolism, pulmonary embolism, arterial embolism, venous embolism, allergic inflammation, cardiovascular disease, graft- related diseases, graft versus host disease (GVHD), disorders associated with graft transplantation rejection, chronic rejection, and tissue or cell allografts or xenografts, autoimmune diseases, degeneration after trauma, stroke, transplant rejection, allergic conditions and hypersensitivity, e.g., allergic rhinitis,
10 allergic eczema and the like, skin diseases, dermal inflammatory disorders, or any combination thereof.

[0506] In one embodiment, the mutant IL-2, fusion protein, nucleic acid, expression vector, composition, pharmaceutical composition or medicament according to the present invention is used alone.

15 [0507] In another embodiment, the mutant IL-2, fusion protein, nucleic acid, expression vector, composition, pharmaceutical composition or medicament according to the present invention is used in combination with at least one further therapeutic agent.

[0508] In one embodiment, for simultaneous administration, the at least one further therapeutic agent and the mutant IL-2, fusion protein, nucleic acid, expression vector,
20 composition, pharmaceutical composition or medicament according to the present invention are administered as one composition or as separate compositions, as appropriate.

[0509] Examples of additional therapeutic agents include, but are not limited to, chemotherapeutic agents, targeted cancer therapy, radiotherapy, immunotherapeutic
25 agents or anti-cancer immunogens, anti-cancer antibodies, cytotoxic agents, anti-angiogenic agents, cell cycle control/apoptosis regulating agents, hormonal regulating agents, tyrosine kinase inhibitors (TKIs), vaccines, immune checkpoint inhibitors, CAR-T and CAR-NK cell therapies, CAR macrophage therapy, tumor-infiltrating lymphocytes (TIL) therapies, immune engaging biologics, steroids, JAK inhibitors, and other

immunosuppressive and/or anti-inflammatory drugs including corticoids, such as, for example, glucocorticoids.

[0510] In one embodiment, the at least one further therapeutic agent is a therapeutic agent useful for treating the specific disease, disorder or condition to be treated in the present invention.

[0511] For example, for treating cancer, the at least one further therapeutic agent may be selected from the group comprising, but not limited to, chemotherapeutic agents, targeted cancer therapy, radiotherapy, immunotherapeutic agents or anti-cancer immunogens, anti-cancer antibodies, cytotoxic agents, anti-angiogenic agents, cell cycle control/apoptosis regulating agents, hormonal regulating agents, tyrosine kinase inhibitors (TKIs), vaccines, immune checkpoint inhibitors, CAR-T and CAR-NK cell therapies, CAR macrophage therapy, tumor-infiltrating lymphocytes (TIL) therapies, immune engaging biologics, and other immunosuppressive and/or anti-inflammatory drugs selected from corticoids, such as, for example, glucocorticoids.

[0512] For example, for treating inflammatory diseases, the at least one further therapeutic agent may be selected from the group comprising, but not limited to, anti-inflammatory drugs, steroids, and JAK inhibitors.

BRIEF DESCRIPTION OF THE DRAWINGS

[0513] **Figure 1** is a table showing, for fusion proteins comprising a mutant IL-2 according to the present invention fused to a Fab against HER2, their production and purification (concentration in mg/mL, total mass post purification in mg, yield in mg/L, purity by SDS-PAGE), their ability to bind HER2, CD25 and CD122 (Yes or No), their ability to activate CD4 T cells, Treg cells, CD8 T cells and NK cells (mean EC50 in pM), and their ability to be bio-conjugated (Yes or No, NT = not tested). **Figure 1A** presents the results with the fusion proteins comprising 2-102, 2-105 to 2-107, 2-112 to 2-116, and 2-120 IL-2 mutants. **Figure 1B** presents the results with the fusion proteins comprising 2-121 to 2-126, 2-130 and 2-131 IL-2 mutants.

[0514] **Figure 2** is a combination of two graphs showing the percentage of pSTAT5 expression on Treg cells (A) and on CD8 T cells (B) induced by the following fusion proteins: IL2-Fab (*i.e.* a wild type IL-2 fused to a Fab against HER2, used as a control), and the fusion proteins comprising either the 2-114 or the 2-115 IL-2 mutant, a 10 kDa PEG fused to the non-endogenous transglutaminase motif of IL-2 and a Fab against HER2 (5 *i.e.* fusion proteins 2-114-P10 and 2-115-P10, respectively).

[0515] **Figure 3** is a combination of 4 graphs showing the percentage of pSTAT5 expression on CD4 T cells, CD8 T cells, Treg cells and NK cells induced by a fusion protein comprising the 2-120 IL-2 mutant, a 10 kDa PEG fused to the non-endogenous transglutaminase motif of IL-2 and a Fab against HER2 (2-120-P10). The continuous line (10 represents 2-120 and the dashed line represents the construct 2-120-P10.

[0516] **Figure 4** is a combination of 4 graphs showing the percentage of pSTAT5 expression on CD4 T cells, CD8 T cells, Treg cells and NK cells induced by a fusion protein comprising the 2-121 IL-2 mutant, a 10 kDa PEG fused to the non-endogenous transglutaminase motif of IL-2 and a Fab against HER2 (2-121-P10). The continuous line (15 represents 2-121 and the dashed line represents the construct 2-121-P10.

[0517] **Figure 5** is a combination of 4 graphs showing the percentage of pSTAT5 expression on CD4 T cells, CD8 T cells, Treg cells and NK cells induced by the fusion protein comprising the 2-122 IL-2 mutant, a 10 kDa PEG fused to the non-endogenous transglutaminase motif of IL-2 and a Fab against HER2 (2-122-P10). The continuous line (20 represents 2-122 and the dashed line represents the construct 2-122-P10.

[0518] **Figure 6** is a combination of 4 graphs showing the percentage of pSTAT5 expression on CD4 T cells, CD8 T cells, Treg cells and NK cells induced by the fusion protein comprising the 2-123 IL-2 mutant, a 10 kDa PEG fused to the non-endogenous transglutaminase motif of IL-2 and a Fab against HER2 (2-123-P10). The continuous line (25 represents 2-123 and the dashed line represents the construct 2-123-P10.

[0519] **Figure 7** is a combination of 4 graphs showing the percentage of pSTAT5 expression on CD4 T cells, CD8 T cells, Treg cells and NK cells induced by the fusion protein comprising the 2-124 IL-2 mutant, a 10 kDa PEG fused to the non-endogenous

transglutaminase motif of IL-2 and a Fab against HER2 (2-124-P10). The continuous line represents 2-124 and the dashed line represents the construct 2-124-P10.

[0520] **Figure 8** is a combination of 7 graphs showing the percentage of pSTAT5 expression on NK cells (NK92 cells) induced by fusion proteins according to the present invention. % STAT5 is normalized against IL2-Fab. It presents the effects of IL2-Fab (control); and of the following fusion proteins: a fusion protein comprising the 2-114 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-114 and 2-114-P10, respectively) (A), a fusion protein comprising the 2-115 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-115 and 2-115-P10, respectively) (B), a fusion protein comprising the 2-120 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-120 and 2-120-P10, respectively) (C), a fusion protein comprising the 2-121 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-121 and 2-121-P10, respectively) (D), a fusion protein comprising the 2-124 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-124 and 2-124-P10, respectively) (E), a fusion protein comprising the 2-125 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-125 and 2-125-P10, respectively) (F), and a fusion protein comprising the 2-126 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-126 and 2-126-P10, respectively) (G). Unstimulated (Unstim.) represents cells which have not been activated.

[0521] **Figure 9** is a table showing, for fusion proteins comprising a mutant IL-2 according to the present invention (*i.e.* 2-132 to 2-135, 2-137, 2-138 and 2-140 IL-2 mutant) fused to a Fab against HER2, their production and purification (concentration in mg/mL, total mass post purification in mg, yield in mg/L, purity by SDS-PAGE), their ability to bind HER2, CD25 and CD122 (Yes or No), their ability to activate CD4 T cells, Treg cells, CD8 T cells and NK cells (mean EC50 in pM), and their ability to be bio-conjugated (Yes or No, NT = not tested).

[0522] **Figure 10** is a table showing, for fusion proteins comprising a mutant IL-2 according to the present invention fused to a Fab against HER2, their production and purification (concentration in mg/mL, total mass post purification in mg, yield in mg/L, purity by SDS-PAGE), their ability to bind HER2, CD25 and CD122 (Yes or No), their

ability to activate CD4 T cells, Treg cells, CD8 T cells and NK cells (mean EC50 in pM), and their ability to be bio-conjugated (Yes or No, NT = not tested). **Figure 10A** presents the results with the fusion proteins comprising 2-142 to 2-147, and 2-150 to 2-153 IL-2 mutants. **Figure 10B** presents the results with the fusion proteins comprising 2-154 to 2-161 IL-2 mutants.

[0523] **Figure 11** is a table showing, for fusion proteins comprising a mutant IL-2 according to the present invention (*i.e.* 2-162 to 2-164, 2-166, 2-167, 2-170 and 2-171 IL-2 mutant) fused to a Fab against HER2, their production and purification (concentration in mg/mL, total mass post purification in mg, yield in mg/L, purity by SDS-PAGE), their ability to bind HER2, CD25 and CD122 (Yes or No), their ability to activate CD4 T cells, Treg cells, CD8 T cells and NK cells (mean EC50 in pM), and their ability to be bio-conjugated (Yes or No, NT = not tested).

[0524] **Figure 12** is a combination of 3 graphs showing the percentage of pSTAT5 expression on Treg cells induced by IL2-Fab (control); and by the following fusion proteins according to the present invention: a fusion protein comprising the 2-130 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-130-P10 and 2-130, respectively) (A), a fusion protein comprising the 2-135 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-135-P10 and 2-135, respectively) (B), a fusion protein comprising the 2-137 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-137-P10 and 2-137, respectively) (C). Unstimulated (Unstim.) represents cells which have not been activated.

[0525] **Figure 13** is a combination of 3 graphs showing the percentage of pSTAT5 expression on CD8 T cells induced by IL2-Fab (control); and by the following fusion proteins according to the present invention: a fusion protein comprising the 2-130 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-130-P10 and 2-130, respectively) (A), a fusion protein comprising the 2-135 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-135-P10 and 2-135, respectively) (B), a fusion protein comprising the 2-137 IL-2 mutant, a Fab against HER2 and with or without a 10 kDa PEG (2-137-P10 and 2-137, respectively) (C). Unstimulated (Unstim.) represents cells which have not been activated.

[0526] **Figure 14** is a schema showing an example of a fusion protein according to the present invention. The fusion protein of the present invention may or may not have a binding moiety, and may or may not have a linker between the binding moiety and the IL-2 mutant. Regarding the linkers, the linker between the binding moiety and the IL-2 mutant may or may not be proteolytically cleavable, and the linker between the masking moiety and the IL-2 mutant may or may not be cleavable.

[0527] **Figure 15** is a combination of 4 graphs showing the percentage of pSTAT5 expression on CD4 T cells (A), Treg cells (B), CD8 T cells (C) and NK cells (D) induced by IL2-Fab (control); and by a fusion protein comprising the 2-130 IL-2 mutant, a Fab against HER2 and with or without a 40 kDa PEG (2-130-P40 and 2-130, respectively). Unstimulated (Unstim.) represents cells which have not been activated.

[0528] **Figure 16** is a combination of 4 graphs showing the percentage of pSTAT5 expression on CD4 T cells (A), Treg cells (B), CD8 T cells (C) and NK cells (D) induced by IL2-Fab (control); and by a fusion protein comprising the 2-132 IL-2 mutant, a Fab against HER2 and with or without a 40 kDa PEG (2-132-P40 and 2-132, respectively). Unstimulated (Unstim.) represents cells which have not been activated.

[0529] **Figure 17** is a combination of 3 graphs showing the percentage of pSTAT5 expression on NK cells (NK92 cells) induced by IL2-Fab (control); and by a fusion protein comprising the 2-135 IL-2 mutant, a Fab against PD-1 and with or without a 40 kDa PEG (2-135-P40 and 2-135, respectively) (A), a fusion protein comprising the 2-136 IL-2 mutant, a Fab against PD-1 and with or without a 40 kDa PEG (2-136-P40 and 2-136, respectively) (B), a fusion protein comprising the 2-137 IL-2 mutant, a Fab against PD-1 and with or without a 40 kDa PEG (2-137-P40 and 2-137, respectively). % STAT5 is normalized against IL2-Fab.

[0530] **Figure 18** is a graph showing the percentage of pSTAT5 expression on NK cells (NK92 cells) induced by IL2-Fab (control); and by a fusion protein comprising the 2-210 IL-2 mutant, a Fab against HER2 and with or without a 40 kDa PEG (2-210-P40 and 2-210, respectively). % STAT5 is normalized against IL2-Fab.

[0531] **Figure 19** is a graph showing *in vivo* PK study with control fusion proteins (IL2-Fab with a F42 mutation (non-alpha IL2-Fab) or without F42 mutation (WT IL2-Fab), or a fusion protein comprising the 2-132 mutant, a Fab against PD-1 and with a 10 kDa or a 40 kDa PEG.

- 5 [0532] **Figure 20** is a graph showing the percentage of pSTAT5 expression on NK cells (NK92 cells) induced by IL2-Fab (control); and by a fusion protein comprising the 2-222 IL-2 mutant, a Fab against PD-1 and with or without a 40 kDa PEG (2-222-P40 and 2-222, respectively). % STAT5 is normalized against IL2-Fab.

- 10 [0533] **Figure 21** is a graph showing the percentage of pSTAT5 expression on NK cells (NK92 cells) induced by IL2-Fab (control); and by a fusion protein comprising the 2-138 IL-2 mutant, a Fab against PD-1 and with or without a 40 kDa PEG (2-138-P40 and 2-138, respectively). % STAT5 is normalized against IL2-Fab.

EXAMPLES

- 15 [0534] The present invention is further illustrated by the following examples.

Materials and Methods

Expression and purification of immunocytokine constructs

- [0535] DNA coding for the amino acid sequence of the listed constructs were synthesized and cloned into the mammalian transient expression vector plasmid pETE
- 20 V2. All constructs were expressed using CHO based transient expression system and the resulting antibody containing cell culture supernatants were clarified by centrifugation and filtration. All constructs were purified from cell culture supernatants via affinity chromatography. Purified antibody was buffer exchanged into phosphate buffered saline solution. The purity of the antibodies was determined by reducing and denaturing Sodium
- 25 Dodecyl Sulfate Polyacrylamide gels and analyzed by semi-preparative SEC. Antibody concentrations were determined by measuring absorbance at 280nm and calculated theoretical extinction coefficient.

pSTAT5 activation assay on hPBMc

PBMc's were washed and counted into complete RPMI in absence of IL2. Cells were seeded at 1×10^6 /ml and 7×10^5 cells per well and incubated at 37°C for 1hr. Dilutions of the immunocytokines of interest (Table 8) from 1000nM – 0.1pM were prepared in PBS creating a 10x concentration of each sample. 5ul/well of 10x immunocytokine was added to the cells for a final concentration range of 100nM – 0.01pM and incubated at 37°C, 5% CO₂ for 15min. Membrane staining antibodies specific for each individual cell type were added to corresponding wells; anti-CD25-FITC (Biolegend 3561606), anti-CD127-PE (ebio 12-1278-42), anti-CD8-ECD (BC-B737660), anti-CD4-PC7 (BC-B737660), anti-CD3-Pacific Blue (BC-B49204) and anti-CD56-BV510 (Biolegend 318340). For preparation of FACS plate 8ul of R1 fixative reagent was incubated in well at RT for 10min then 65ul FBS and 145ul of Buffer Perm reagent, mixed and incubated at 37°C for 5 min. The plate was centrifuged at 400g for 2min prior to adding 50ul/well of intercellular staining antibody (pSTAT5-AF647-FL6). The plate was incubated at RT for 30min in dark then washed once with 150ul R4 buffer wash reagent. After washing each sample was resuspended in 150ul of buffer R4 and transferred to a microtitre tube and read on the Navios EX.

Receptor binding affinity assay

A capture assay set up was utilised for receptor binding analysis. Anti-human IgG (Cat No. BR100839) was coupled to CM5 chips (GE Healthcare) using standard amine chemistry to ~ 9000RU. Human IL-2R α -Fc at 1 μ g/ml in HBS-P+ running buffer was captured to ~60RU on flow cell 2. Human IL-2R γ -Fc at 1 μ g/ml in HBS-P+ running buffer was captured to ~120RU on flow cell 1. Her2 binding of the Fab portion was assessed in the same assay set up with Her2-Fc captured on the chip and analyte run over the surface. A four point 3-fold dilution series for each analyte (Immunocytokine) was used for single cycle kinetic analysis. An analyte concentration range of 66.6nM – 1800nM was used for both IL-2R α and IL-2R γ and a range of 2.5nM – 66.6nM for HER2 binding. Between each run the chip was regenerated with 3M MgCl₂. A single cycle kinetic protocol was run on Biacore T200 and 1:1 kinetic analysis or steady state affinity measurement

following double reference-subtraction was performed using Biacore T200 evaluation software V 2.0.1.

A similar protocol was used when testing both masked and unmasked immunocytokines

pK assay

- 5 A Nunc MaxiSorp was coated with 25 ul/well of anti-Nivolumab Fab antibody (BioRad-HCA299 Human Fab) protein at 2 µg/ml in coating buffer (PBS1x). The plate was sealed and incubated overnight at 4°C. The sample wells were blocked with PBS w/v 1% BSA for 1 hour at room temperature followed by washing 3 x PBS-0.05% Tween-20. Serum samples collected from test groups were prepared and added to the plate prior to
- 10 incubation at room temperature for 2 hours and subsequent washing 3 x PBS-0.05% Tween-20. Detection antibody, anti- kappa light chain HRP antibody (Novus NB500-331H) was added to the plate with incubation and washing steps as detailed above. This was followed by the addition of the detection sustrate (TMB) and optical absorbance measured at 450nm.

15 **Results**

Example 1: Mutant IL-2 with at least one non-endogenous transglutaminase motif

- [0536] Fusion proteins, comprising a mutant IL-2 with at least one non-endogenous transglutaminase motif and a Fab against HER2, were constructed and studied regarding different parameters: i) their ability to be produced and purified, ii) their ability to bind
- 20 HER2, CD25 and CD122, iii) their ability to activate CD4 T cells, Treg cells, CD8 T cells and NK cells, and iv) their ability to be bio-conjugated with a range of PEG moieties of varying sizes and structures including, but not limited to, 10kDa linear and 40kDa branched. Results are disclosed in the **Figure 1**.

- [0537] All the constructs comprising a mutant IL-2 having at least one non-endogenous
- 25 transglutaminase motif i) can be robustly produced and purified, ii) bind to HER2 and CD122 (IL-2Rβ), iii) are able to activate CD4 T cells, CD8 T cells, and NK cells, and iv) are able to be bio-conjugated with a PEG. All the fusion proteins except the ones

comprising 2-106, 2-107, 2-130 and 2-131 IL-2 mutants are able to bind to CD25 (IL-2R α), while the fusion proteins comprising 2-106, 2-107, 2-130 and 2-131 IL-2 mutants do not bind to CD25 and therefore exhibit reduced potency in activation of Treg cells.

[0538] In addition, fusion proteins comprising a mutant IL-2 with at least one non-
5 endogenous transglutaminase motif as described herein, a PEG fused to the IL-2 mutant by transglutamination with a linker, and a Fab against HER2 were also generated, and their ability to activate Treg cells, NK cells and CD8 T cells was evaluated. As shown in **Figure 2**, the presence of a 10 kDa PEG fused to the 2-114 or the 2-115 IL-2 mutant prevents the activation of CD8 T cells and has a lower impact on Treg cell activation. The
10 same results were obtained with the 2-125 and the 2-126 mutants (data not shown).

[0539] **Figures 3-7** show that the presence of a PEG fused to the 2-120, 2-121, 2-122, 2-123 or 2-124 IL-2 mutant prevents the activation of CD4 T cells, and CD8 T cells, and has a lower impact on activation of NK cells and Treg cells. **Figure 8** further shows that the presence of a PEG fused to the 2-114, 2-115, 2-120, 2-121, 2-124, 2-125 or the 2-126
15 IL-2 mutant impact NK cells activation to a different extent depending on the IL-2 mutant. Regarding the 2-130 IL-2 mutant, as shown in **Figures 12, 13 and 15**, the presence of a 10 kDa PEG fused to this IL-2 mutant strongly prevents the activation of both CD8 T cells and Treg cells, and the presence of a 40 kDa PEG prevents the activation of CD4 and CD8 T cells as well as the activation of Treg cells and NK cells.

20 [0540] **Figure 18** shows that the presence of a 40kDa PEG fused to the 2-210 IL-2 mutant impacts NK cells activation.

[0541] Thus, these data show that a fusion protein comprising a mutant IL-2 having a PEG fused to a non-endogenous transglutaminase motif prevents the activation of CD4 T cells and CD8 T cells, and, depending on the IL-2 mutant comprised in the fusion protein,
25 impact NK cells and Treg cells activation to a different extent.

[0542] Altogether, these data show that fusing a PEG as a masking moiety to an IL-2 mutant with at least one non-endogenous transglutaminase motif by transglutamination enables to prevent the IL-2 signaling on cells. This masking is reversible by the use of a cleavable linker between the PEG and the IL-2, which, under the suitable conditions, will

be cleaved and will induce the releasing of the IL-2.

Example 2: Mutant IL-2 with at least one non-endogenous transglutaminase motif and a F42A mutation

[0543] Fusion proteins, comprising a mutant IL-2 with at least one non-endogenous
5 transglutaminase motif and a F42A mutation, fused to a Fab against HER2, were
constructed and studied regarding different parameters: i) their ability to be produced and
purified, ii) their ability to bind HER2, CD25 and CD122, iii) their ability to activate CD4
T cells, CD8 T cells, Treg cells and NK cells, and iv) their ability to be bio-conjugated
with a PEG. Results are disclosed in the **Figure 9**.

10 [0544] All the constructs comprising IL-2 mutants having at least one non-endogenous
transglutaminase motif and a F42A mutation i) can be robustly produced and purified, ii)
bind to HER2 and CD122 but not to CD25, iii) are able to activate CD4 T cells, CD8 T
cells, Treg cells and NK cells, and iv) are able to be bio-conjugated with a PEG.

[0545] In addition, fusion proteins comprising a mutant IL-2 with at least one non-
15 endogenous transglutaminase motif and a F42A mutation, a PEG fused to the IL-2 mutant
by transglutamination with a linker, and a Fab against HER2 were also generated, and
their ability to activate Treg cells and CD8 T cells was evaluated. As shown in **Figure
12**, the presence of a 10 kDa PEG fused to the 2-135 or the 2-137 IL-2 mutant prevents
the activation of CD8 T cells and, depending on the IL-2 mutant, impacts Treg cells
20 activation to a different extent.

[0546] Furthermore, as shown in **Figure 16**, the presence of a 40 kDa PEG fused to the
2-132 mutant prevents the activation of CD4 T cells, CD8 T cells, Treg cells and NK
cells, with a lower impact on Treg cells and NK cells activation.

[0547] Fusion proteins comprising a mutant IL-2 with at least one non-endogenous
25 transglutaminase motif and a F42A mutation, a 40 kDa PEG fused to the IL-2 mutant by
transglutamination with a linker, and a Fab against PD-1 were also generated, and their
impact on NK cells activation was evaluated. As demonstrated in **Figures 17, 20 and 21**,
the presence of a 40 kDa PEG fused to the 2-135, 2-136, 2-137, 2-138 or the 2-222 mutant

affects NK cells activation.

[0548] *In vivo* PK study was also performed with fusion proteins comprising a mutant IL-2 with at least one non-endogenous transglutaminase motif and a F42A mutation (*i.e.* 2-132 mutant), a 10 kDa or 40 kDa PEG fused to the IL-2 mutant by transglutamination
5 with a linker, and a Fab against PD-1. As shown in **Figure 19**, the fusion protein with a branched 40kD PEG has an improved PK over the fusion protein with a linear 10kD PEG. However, both masked fusion proteins demonstrate improved PK over the control fusion proteins.

[0549] Thus, these data show that a fusion protein comprising a mutant IL-2 having a
10 PEG fused to a non-endogenous transglutaminase motif and a F42A mutation prevents the activation of CD8 T and CD4 T cells, and, depending on the IL-2 mutant comprised in the fusion protein, impact Treg cells and NK cells activation to a different extent.

[0550] In addition, these data show that the addition of PEG improves PK of the fusion proteins *in vivo*.

15 Example 3: Mutant IL-2 with at least two non-endogenous transglutaminase motifs

[0551] Fusion proteins, comprising a mutant IL-2 with at least two non-endogenous transglutaminase motifs, fused to a Fab against HER2, were constructed and studied regarding different parameters: i) their ability to be produced and purified, ii) their ability to bind HER2, CD25 and CD122, and iii) their ability to activate CD4 T cells, Treg cells
20 and NK cells. Results are disclosed in the **Figures 10A and 10B**.

[0552] All the constructs comprising IL-2 mutants having at least two non-endogenous transglutaminase motifs i) can be robustly produced and purified, ii) bind to HER2 and CD122 but not to CD25, and iii) are able to activate CD4 T cells, CD8 T cells, Treg cells and NK cells.

25 [0553] Thus, these data show that it is possible to combine two non-endogenous transglutaminase motifs to obtain functional IL-2 mutants and fusion proteins.

Example 4: Mutant IL-2 with at least two non-endogenous transglutaminase motifs, one of the motifs being the sequence LLQFKF (SEQ ID NO: 10) with a Q41 residue

[0554] Fusion proteins, comprising a mutant IL-2 with at least two non-endogenous transglutaminase motifs with one of the motifs being the sequence LLQFKF (SEQ ID NO: 10) with a Q41 residue, fused to a Fab against HER2, were constructed and studied regarding different parameters: i) their ability to be produced and purified, ii) their ability to bind HER2, CD25 and CD122, iii) their ability to activate CD4 T cells, Treg cells and NK cells, and iv) their ability to be bio-conjugated with a PEG. Results are disclosed in the **Figure 11**.

10 [0555] All the constructs comprising IL-2 mutants having at least two non-endogenous transglutaminase motifs with one of the motifs being the sequence LLQFKF (SEQ ID NO: 10) with a Q41 residue i) can be robustly produced and purified, ii) bind to HER2 and CD122 and CD25, iii) are able to activate CD4 T cells, CD8 T cells and NK cells, but not Treg cells, and iv) are able to be bio-conjugated with a PEG (among the ones
15 tested).

[0556] Thus, these data show that it is possible to combine two non-endogenous transglutaminase motifs with one of the motifs being the sequence LLQFKF (SEQ ID NO: 10) with a Q41 residue, to obtain functional IL-2 mutants and fusion proteins.

CLAIMS

1. A mutant IL-2 derived from human IL-2 with the sequence SEQ ID NO: 1 or from a variant thereof, comprising at least one non-endogenous transglutaminase motif as compared to SEQ ID NO: 1 or variant thereof, wherein said motif is inserted by:
- 5 - insertion of a Q residue at one or more of the following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, T51, L53, Q57, H79, E95, E100, E110 and S130, and/or
- insertion of a motif at the N-terminus or C-terminus, wherein said motif comprises a sequence selected from the group comprising or consisting of
- 10 LQS and TQG,
- optionally wherein the mutant IL-2 comprises, by reference to SEQ ID NO: 1, (i) a substitution of the cysteine residue 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation), (ii) a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation), or (iii) both a substitution of the cysteine residue
- 15 125 with any other amino acid residue, preferably a serine residue (*i.e.*, a C125S mutation) and a substitution of the glutamine 74 residue with any other amino acid residue, preferably a glutamate residue (*i.e.*, a Q74E mutation).
2. The mutant IL-2 according to claim 1, wherein said at least one non-endogenous transglutaminase motif is inserted by insertion of a Q residue at one or more of the
- 20 following positions by reference to SEQ ID NO: 1: H16, L18, L19, N26, K32, N33, T41, F42, Q57, H79, E95, E100, S130.
3. The mutant IL-2 according to claim 1 or claim 2, wherein said at least one non-endogenous transglutaminase motif comprises or consists of a sequence selected
- 25 from the group comprising or consisting of X₁LQ wherein X₁ is A, L, H, I or V; NX₂Q wherein X₂ is Y, H or F; YRQ, LTQ; AQX₃ wherein X₃ is A or E, TEQ, LFQ; GSQ and VIQ, preferably comprising or consisting of X₁LQ wherein X₁ is L, H, I or V; NX₂Q wherein X₂ is Y, H or F; YRQ, LTQR (SEQ ID NO: 2); LFQ; GSQ and VIQ.

4. The mutant IL-2 according to any one of claims 1 to 3, wherein said at least one non-endogenous transglutaminase motif comprises or consists of:
- a sequence selected from the group comprising or consisting of
 5 LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD
 (SEQ ID NO: 5), MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO:
 7), NNYQNP (SEQ ID NO: 8), NNHQNP (SEQ ID NO: 9), LLQFKF
 (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO:
 12), LLQC (SEQ ID NO: 13), KNFQLRPRD (SEQ ID NO: 14),
 10 KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), VLQ,
 LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ,
 NNYRQP (SEQ ID NO: 102), NRYRQP (SEQ ID NO: 103), MLTQGF
 (SEQ ID NO: 104), LAQELK (SEQ ID NO: 105), LAQALK (SEQ ID
 NO: 106), KATEQK (SEQ ID NO: 107), EYALQ (SEQ ID NO: 108) and
 DYALQ (SEQ ID NO: 109), preferably comprising or consisting of
 15 LEHLLQD (SEQ ID NO: 3), LEHLQSD (SEQ ID NO: 4), LLQLLLD
 (SEQ ID NO: 5), MILQGIN (SEQ ID NO: 6), MLLQGIN (SEQ ID NO:
 7), NNYQNP (SEQ ID NO: 8), NNHQNP (SEQ ID NO: 9), LLQFKF
 (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO:
 12), LLQC (SEQ ID NO: 13), KNFQLRPRD (SEQ ID NO: 14),
 20 KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), VLQ,
 LLQ, GSQTT (SEQ ID NO: 17), GSQST (SEQ ID NO: 18), VIQ,
 NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103), and/or
 - a sequence selected from the group comprising or consisting of
 25 LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), TQGAPTSSSTKKTQ (SEQ
 ID NO: 41), LQSPTSSSTKKTQ (SEQ ID NO: 42), TQGASSSTKKTQ
 (SEQ ID NO: 43), TQGASSTKKTQ (SEQ ID NO: 44), and
 TQGASTKKTQ (SEQ ID NO: 45), preferably wherein said sequence is
 added at the N-terminus, and/or
 - a sequence selected from the group comprising or consisting of GGTQGA
 30 (SEQ ID NO: 46), GGLQSP (SEQ ID NO: 47), GLQSP (SEQ ID NO:
 48), LQSP (SEQ ID NO: 49) and QGA, preferably wherein said sequence
 is added at the C-terminus.

5. The mutant IL-2 according to any one of claims **1** to **4**, wherein said at least one non-endogenous transglutaminase motif is inserted by:
- insertion of a Q residue at one or more of the following positions by reference to SEQ ID NO: 1: N33, T41, F42, H79, E100, and /or
 - 5 - insertion of a motif at the N-terminus or C-terminus, wherein said motif comprises or consists of a sequence selected from the group comprising or consisting of LQS and TQG.
6. The mutant IL-2 according to any one of claims **1** to **5**, wherein said at least one non-endogenous transglutaminase motif comprises or consists of:
- 10 - a sequence selected from the group comprising or consisting of LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), GSQST (SEQ ID NO: 18), NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103), and/or
 - 15 - a sequence selected from the group comprising or consisting of LQS and TQG, preferably wherein said sequence is added at the C-terminus or at the N-terminus.
7. The mutant IL-2 according to any one of claims **1** to **6**, wherein said at least one non-endogenous transglutaminase motif comprises or consists of:
- 20 - a sequence selected from the group comprising or consisting of LLQFKF (SEQ ID NO: 10), LLQAKF (SEQ ID NO: 11), MLTQRF (SEQ ID NO: 12), KLFQLRPRD (SEQ ID NO: 15), KLLQLRPRD (SEQ ID NO: 16), GSQST (SEQ ID NO: 18), NNYRQP (SEQ ID NO: 102) and NRYRQP (SEQ ID NO: 103), and/or
 - 25 - a sequence selected from the group comprising or consisting of LQSPGAPTSSSTKKTQ (SEQ ID NO: 40), LQSPTSSSTKKTQ (SEQ ID NO: 42), and TQGASSTKKTQ (SEQ ID NO: 44), preferably wherein said sequence is added at the N-terminus, and/or

- a sequence selected from the group comprising or consisting of GGLQSP (SEQ ID NO: 47), GLQSP (SEQ ID NO: 48), LQSP (SEQ ID NO: 49) and QGA, preferably wherein said sequence is added at the C-terminus.
- 5
8. The mutant IL-2 according to any one of claims **1** to **7**, wherein the mutant comprises or consists of a sequence selected from the group comprising or consisting of SEQ ID NO: 30, SEQ ID NO: 20, SEQ ID NO: 19, SEQ ID NO: 21 to SEQ ID NO: 29, SEQ ID NO: 31 to SEQ ID NO: 39, SEQ ID NO: 51 to SEQ ID NO: 63, SEQ ID NO: 110 to SEQ ID NO: 117, more preferably comprising or consisting of SEQ ID NO: 19 to SEQ ID NO: 39, SEQ ID NO: 51 to SEQ ID NO: 63 and SEQ ID NOs: 110 and 111.
- 10
9. The mutant IL-2 according to any one of claims **1** to **8**, wherein the mutant comprises or consists of a sequence selected from the group comprising or consisting of SEQ ID NO: 26 to SEQ ID NO: 28, SEQ ID NO: 30 to SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, and SEQ ID NO: 58 to SEQ ID NO: 63 and SEQ ID NOs: 110 and 111.
- 15
10. The mutant IL-2 according to any one of claims **1** to **9**, wherein the mutant IL-2 further comprises a substitution of the F42 residue by reference to SEQ ID NO: 1 into any other amino acid, more preferably a substitution of the F42 residue into an alanine residue.
- 20
11. A fusion protein comprising a mutant IL-2 according to any one of claims **1** to **10**, and further comprising at least one masking moiety, wherein the at least one masking moiety is linked to a Q residue of the at least one non-endogenous transglutaminase motif of the mutant IL-2 with a cleavable linker.
- 25
12. The fusion protein according to claim **11**, wherein the masking moiety is a polyethylene glycol (PEG), preferably a linear or a branched PEG.
13. The fusion protein according to any one of claims **11** to **12**, wherein the fusion protein further comprises an antibody or antigen-binding fragment thereof or an

antibody mimetic, preferably wherein the antibody or antigen-binding fragment thereof or antibody mimetic is linked to the mutant IL-2 with a linker.

- 14.** The fusion protein according to any one of claims **11** to **13**, for use as a medicament.
- 15.** The fusion protein according to any one of claims **11** to **14**, for use in treating a
5 cancer or an inflammatory disease.