

Cytokine therapy is an effective strategy for stimulating the immune system to induce anti-tumor cytotoxicity. In particular, aldesleukin, a recombinant form of interleukin-2 (IL-2), has been approved by the FDA for the treatment of metastatic renal cell carcinoma and melanoma. Unfortunately, cytokines that are administered to patients generally have a very short half-life, thereby requiring frequent dosing. For instance, the product label of aldesleukin, marketed under the brand name Proleukin, states that the drug was shown to have a half-life of 85 minutes in patients who received a 5- minute intravenous (IV) infusion. In addition, administration of high doses of cytokine can cause adverse health outcomes, such as vascular leakage, through systemic immune activation. These findings illustrate the need for developing IL-2 cytokine therapeutics that effectively target tumors without the side effects associated with systemic immune activation.

Provided herein are masked IL-12 cytokines, cleavage products of said masked IL-12 cytokines, and compositions thereof and methods of use thereof for addressing this need.

SUMMARY

The disclosed ~~invention~~disclosure relates to IL-12 cytokines or functional fragments thereof that are engineered to be masked by a masking moiety at one or more receptor binding site(s) of the IL-12 cytokine or functional fragment thereof. The IL-12 cytokines are engineered to be activatable by a protease at a target site, such as in a tumor microenvironment, by including a proteolytically cleavable linker. In the masked cytokine construct, the masking moiety reduces or prevents binding of the IL-12 cytokine or functional fragment thereof to its cognate receptor. Upon proteolytic cleavage of the cleavable linker at the target site, the IL-12 cytokine or functional fragment thereof becomes activated, which renders it capable or more capable of binding to its cognate receptor.

~~In one aspect, the present disclosure provides~~ herein ~~is~~ a masked IL-12 cytokine comprising a protein heterodimer comprising:

a first polypeptide chain comprising:

N' **HL1-L1-MM** C'

and a second polypeptide chain comprising:

N' **HL2-L2-C** C'

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-12 cytokine or functional fragment thereof,

wherein the first half-life extension domain is associated with the second half-life extension domain, and

wherein one of the first linker or the second linker comprises a proteolytically cleavable peptide.

In some embodimentexamples, the IL-12 polypeptide or functional fragment thereof comprises an IL-12p40 polypeptide or functional fragment thereof covalently linked to an IL-12p35 polypeptide or functional fragment thereof.

5 In some embodimentexamples, the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length.

In some embodimentexamples, the IL-12p40 – IL-12p35 linker is rich in amino acid residues G and S.

In some embodimentexamples, the IL-12p40 – IL-12p35 linker comprises SEQ ID NO: 3.

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In some embodimentexamples, the IL-12p40 polypeptide comprises SEQ ID NO: 1 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 1.

In some embodimentexamples, the IL-12p40 polypeptide comprises SEQ ID NO: 1.

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In some embodimentexamples, the IL-12p40 polypeptide comprises at least one amino acid modification to the GAG-binding domain (KSKREKKDRV) as compared to the amino acid sequence of SEQ ID NO: 1.

In some embodimentexamples, the IL-12p40 polypeptide comprises SEQ ID NO: 57.

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In some embodimentexamples, the IL-12p40 polypeptide comprises SEQ ID NO: 58.

In some embodimentexamples, the IL-12p40 polypeptide comprises an amino acid sequence having one or more cysteine substitution mutations as compared to the amino acid sequence of SEQ ID NO: 1.

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In some embodimentexamples, the IL-12p40 polypeptide comprises SEQ ID NO: 59.

In some embodimentexamples, the IL-12p40 polypeptide comprises SEQ ID NO: 60.

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In some embodimentexamples, the IL-12p35 polypeptide comprises SEQ ID NO: 2 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodimentexamples, the IL-12p35 polypeptide comprises SEQ ID NO: 2.

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In some embodimentexamples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 4.

In some embodimentexamples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 61.

In some embodimentexamples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 62.

In some embodimentexamples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 63.

In some embodimentexamples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 64.

In some embodimentexamples, the masking moiety comprises an IL-12 cytokine receptor, or a subunit or functional fragment thereof.

In some embodimentexamples, the masking moiety comprises the extracellular domain of human IL-12R β 1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

In some embodimentexamples, the masking moiety comprises residues 24 to 237 of human IL-12R β 1, namely a sequence having SEQ ID NO: 5.

In some embodimentexamples, the masking moiety comprises residues 24 to 545 of human IL-12R β 1, namely a sequence having SEQ ID NO: 6.

In some embodimentexamples, the masking moiety comprises the extracellular domain of human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

In some embodimentexamples, the masking moiety comprises residues 24 to 212 of human IL-12R β 2, namely a sequence having SEQ ID NO: 7.

In some embodimentexamples, the masking moiety comprises residues 24 to 222 of human IL-12R β 2, namely a sequence having SEQ ID NO: 8, or the masking moiety comprises residues 24 to 227 of human IL-12R β 2, namely a sequence having SEQ ID NO: 11.

In some embodimentexamples, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9.

In some embodimentexamples, the masking moiety comprises at least one amino acid modification as compared to the sequence of SEQ ID NO: 9, optionally wherein said modifications are cysteine substitution mutations.

In some embodimentexamples, the masking moiety comprises SEQ ID NO: 65.

In some embodimentexamples, the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely a sequence having SEQ ID NO: 10.

In some [embodimentexamples](#), the cleavable peptide is from 6 to 10 amino acids in length.

In some [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 15.

5 In some [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 41.

In some [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 42.

In some [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 43.

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In some [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 44.

In some [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 45.

15 In some [embodimentexamples](#), the first polypeptide chain comprises:

N' **HL1-non-cleavable L1-MM** C'

and the second polypeptide chain comprises

N' **HL2-cleavable L2-C** C'

20 In some [embodimentexamples](#), the non-cleavable linker is between 3 and 18 amino acids in length.

In some [embodimentexamples](#), the non-cleavable linker is between 3 and 15 amino acids in length.

In some [embodimentexamples](#), the non-cleavable linker is rich in amino acid residues G and S.

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In some [embodimentexamples](#), the non-cleavable linker includes $[(G)_nS]$, where $n=4$ or 5 .

In some [embodimentexamples](#), the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12.

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In some [embodimentexamples](#), the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13.

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In some [embodimentexamples](#), the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some [embodimentexamples](#), the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 54 (GGSGGSGGSGGSGGSSGP).

In some [embodimentexamples](#), the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 55 (PGGSGP).

In some [embodimentexamples](#), the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 56 (GGSPG).

In some [embodimentexamples](#), the cleavable linker comprises a proteolytically cleavable peptide (CP) flanked on both sides by a spacer domain (SD):

SD1-CP-SD2

where SD1 and SD2 are different, such that the first polypeptide chain comprises:

N' **HL1-non-cleavable L1-MM** C'

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2** -C C'

In some [embodimentexamples](#), the first spacer domain (SD1) is between 3 and 10 amino acids in length.

In some [embodimentexamples](#), SD1 comprises SEQ ID NO: 16.

In some [embodimentexamples](#), SD1 comprises SEQ ID NO: 17.

In some [embodimentexamples](#), the second spacer domain (SD2) is between 3 and 6 amino acids in length.

In some [embodimentexamples](#), SD2 comprises SEQ ID NO: 18.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 44 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 45 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.

In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 19.

In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 20.

In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 46. (GGSGGSMPYDLYHPSGP)

5 In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 47.
(GGSGSGSGSMPYDLYHPSGP)

In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 48. (GGSGGSDSGGFMLTSGP)

10 In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 49.
(GGSGSGSGSDSGGFMLTSGP)

In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 50. (GGSGGSRAAAVKSPSGP)

15 In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 51.
(GGSGSGSGSRAAAVKSPSGP)

In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 52. (GGSGGSISSGLLSGRSSGP)

20 In some [embodimentexamples](#), the cleavable linker comprises SEQ ID NO: 53.
(GGSGSGGSISSGLLSGRSSGP)

In some [embodimentexamples](#), the first half-life extension domain comprises a first IgG1 Fc domain or a
25 fragment thereof and the second half-life extension domain comprises a second IgG1 Fc domain or a fragment
thereof.

In some [embodimentexamples](#), the first and/ or second Fc domains each contain one or more modifications that
promote the non-covalent association of the first and the second half-life extension domains.

30 In some [embodimentexamples](#), the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S;
L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C,
T366W and N297A).

In some [embodimentexamples](#), the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S;
35 L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28
(S354C, T366W, N297A and I253A).

In some embodimentexamples, the first polypeptide chain comprises an amino acid sequence of SEQ ID NO:34 and a second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 40.

In another aspect, the present disclosure p~~P~~rovides herein ~~is~~ a cleavage product capable of binding to IL-12R, the cleavage product comprising an IL-12 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in a masked IL-12 cytokine as defined in of any one of the statements or embodimentexamples described herein.

Provided herein is a cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising a polypeptide comprising:

PCP-SD2-C

wherein PCP is a portion of a proteolytically cleavable peptide; SD2 is a spacer domain; and C is an IL-12 cytokine or functional fragment thereof.

In some embodimentexamples, PCP is a portion of a proteolytically cleavable peptide as described herein.

In some embodimentexamples, SD2 is a spacer domain as described herein.

In some embodimentexamples, C is an IL-12 cytokine or functional fragment thereof as described herein.

In some embodimentexamples, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 29.

In some embodimentexamples, the cleavage product comprises an amino acid sequence of SEQ ID NO: 29.

In another aspect, the present disclosure p~~P~~rovides herein ~~is~~ a nucleic acid encoding any one of the masked IL-12 cytokines described herein.

In another aspect, the present disclosure p~~P~~rovides herein ~~is~~ a nucleic acid encoding one of the chains of any one of the masked IL-12 cytokines described herein.

In another aspect, the present disclosure p~~P~~rovides herein ~~is~~ a vector comprising a nucleic acid described herein.

Provided herein is a vector comprising a nucleic acid encoding a masked IL-12 cytokine described herein.

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Provided herein is a vector comprising a nucleic acid encoding one of the chains of a masked IL-12 cytokine described herein.

In another aspect, the present disclosure provides a host cell comprising nucleic acid encoding the masked IL-12 cytokine as described herein. Provided herein is a host cell comprising a nucleic acid described herein.

In one ~~embodiment~~example, the host cell is a HEK cell. In another ~~embodiment~~example, the host cell is a CHO cell.

In another aspect, the present disclosure provides herein ~~is~~ a composition comprising any one of the masked IL-12 cytokines described herein.

In another aspect, the present disclosure provides herein ~~is~~ a pharmaceutical composition comprising any one of the masked IL-12 cytokines described herein and a pharmaceutically acceptable carrier.

In some ~~embodiment~~examples, the pharmaceutical composition is in single unit dosage form.

In some ~~embodiment~~examples, the pharmaceutical composition is formulated for intravenous administration and is in single unit dosage form.

In some ~~embodiment~~examples, the pharmaceutical composition is formulated for injection and is in single unit dosage form.

In some ~~embodiment~~examples, the pharmaceutical composition is a liquid and is in single unit dosage form.

Provided herein is a kit comprising a masked IL-12 cytokine as described herein, or a composition described herein, or a pharmaceutical composition described herein.

In another aspect, the present disclosure provides herein ~~is~~ a method of producing a masked IL-12 cytokine as described herein comprising culturing a host cell described herein under a condition that produces the masked IL-12 cytokine.

Provided herein is a nucleic acid encoding a cleavage product described herein.

Provided herein is a composition comprising a cleavage product described herein.

Provided herein is a pharmaceutical composition comprising a cleavage product described herein, and a pharmaceutically acceptable carrier.

In a further aspect, the present disclosure provides a use of a masked IL-12 cytokine as defined herein in the manufacture of a medicament for treating cancer.

Provided herein is a masked IL-12 cytokine described herein for use in medicine.

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Provided herein is a cleavage product described herein for use in medicine.

Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine described herein.

Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a composition described herein.

5 Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition described herein.

10 Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine described herein, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product described herein.

Provided herein is a method of treating or preventing cancer in a subject, the method comprising a step of producing a cleavage product *in vivo* that is capable of binding to its cognate receptor, wherein the cleavage product is described herein.

15 In some ~~embodiment~~examples, the cancer is a solid tumor.

Provided herein is a masked IL-12 cytokine described herein for use in treating or preventing cancer.

20 Provided herein is a masked IL-12 cytokine described herein for use in a method of treating or preventing cancer, the method comprising administering to the subject an effective amount of the masked IL-12 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as described herein.

25 In some ~~embodiment~~examples, the cancer is a solid tumor.

Provided herein is a cleavage product described herein for use in treating or preventing cancer.

30 Provided herein is a cleavage product described herein for use in a method of treating or preventing cancer, the method comprising a step of administering a masked cytokine described herein to a patient, thereby producing the cleavage product by proteolytic cleavage of the masked cytokine *in vivo*.

35 Provided herein is a cleavage product described herein for use in a method of treating or preventing cancer in a subject, the method comprising a step of producing the cleavage product by *in vivo* proteolytic cleavage from a masked cytokine described herein that has been administered to the subject.

In some ~~embodiment~~examples, the cancer is a solid tumor.

Provided herein is a pharmaceutical composition described herein for use in treating or preventing cancer.

In some embodiment examples, the cancer is a solid tumor.

5 A BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the structure of exemplary embodiments of a masked cytokine that includes a masking moiety, a cytokine or functional fragment thereof (“cytokine”), a half-life extension domain, and a first linker that includes a first cleavable peptide (“1CP”), a first N-terminal spacer domain (“1NSD”), and a first C-terminal spacer domain (“1CSD”). These exemplary embodiments also include a second linker that includes a second cleavable peptide (“2CP”), a second N-terminal spacer domain (“2NSD”), and a second C-terminal spacer domain (“2CSD”). As shown by the arrows, while the exemplary embodiments shows the masking moiety linked to the first linker, and the cytokine or functional fragment thereof is linked to the first linker and the second linker, the masking moiety and the cytokine or functional fragment thereof can be interchanged such that the cytokine or functional fragment thereof is linked to the first linker, and the masking moiety is linked to the first linker and the second linker. **FIG. 1** shows the structure of an exemplary embodiment of a masked cytokine as a monomer.

FIG. 2 shows the structure of an exemplary embodiment of a masked cytokine that includes a masking moiety, a cytokine or functional fragment thereof (“cytokine”), a first half-life extension domain, and a second half-life extension domain. The exemplary embodiment shown in **FIG. 2** also includes a first linker that includes a first cleavable peptide (“1CP”), a first N-terminal spacer domain (“1NSD”), and a first C-terminal spacer domain (“1CSD”), and a second linker that includes a second cleavable peptide (“2CP”), a second N-terminal spacer domain (“2NSD”), and a second C-terminal spacer domain (“2CSD”). The exemplary first and second half-life extension domains include “knobs into holes” modifications that promote the association of the first half-life extension domain with the second half-life extension domain, as shown by the “hole” in the first half-life extension domain and the “knob” in the second half-life extension domain. The first half-life extension domain and the second half-life extension domain are also shown as associating, at least in part, due to the formation of disulfide bonds. It is to be understood that although the “hole” is depicted as part of the first half-life extension domain (linked to the masking moiety) and the “knob” is depicted as part of the second half-life extension domain (linked to the cytokine), the “hole” and the “knob” can alternatively be included in the second half-life extension domain and the first half-life extension domain, respectively, so that the “hole” is a part of the second half-life extension domain (linked to the cytokine) and the “knob” is part of the first half-life extension domain (linked to masking moiety).

FIGs. 3A-3B show exemplary embodiments of masked cytokines prior to (left) and after (right) cleavage

CLAIMS

1. A masked IL-12 cytokine comprises a protein heterodimer comprising:
a first polypeptide chain comprising:

N' **HL1-L1-MM** C'

and a second polypeptide chain comprising:

N' **HL2-L2-C** C'

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-12 cytokine or functional fragment thereof,

wherein the first half-life extension domain is associated with the second half-life extension domain, and

wherein one of the first linker or the second linker comprises a proteolytically cleavable peptide.

2. The masked cytokine of claim 1, wherein the IL-12 polypeptide or functional fragment thereof comprises an IL-12p40 polypeptide or functional fragment thereof covalently linked to an IL-12p35 polypeptide or functional fragment thereof.
3. The masked cytokine of claim 2, wherein the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length.
4. The masked cytokine of claim 2 or 3, wherein the IL-12p40 – IL-12p35 linker is rich in amino acid residues G and S.

~~5. The masked cytokine of any one of claims 2-4, wherein the IL-12p40 – IL-12p35 linker comprises SEQ ID NO: 3. (GGGSGGGSGGGGS)~~

~~6. The masked cytokine of any one of claims 2-5, wherein the IL-12p40 polypeptide comprises SEQ ID NO: 1 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 1.~~

~~7. The masked cytokine of claim 6, wherein IL-12p40 polypeptide comprises SEQ ID NO: 1.~~

- ~~8. The masked cytokine of claim 6, wherein IL-12p40 polypeptide comprises at least one amino acid modification to the GAG-binding domain (KSKREKKDRV) as compared to the amino acid sequence of SEQ ID NO: 1.~~
- ~~9. The masked cytokine of claim 8, wherein IL-12p40 polypeptide comprises SEQ ID NO: 57.~~
- ~~10. The masked cytokine of claim 8, wherein IL-12p40 polypeptide comprises SEQ ID NO: 58.~~
- ~~11. The masked cytokine of any one of claims 6-10, wherein IL-12p40 polypeptide comprises an amino acid sequence having one or more cysteine substitution mutations as compared to the amino acid sequence of SEQ ID NO: 1.~~
- ~~12. The masked cytokine of claim 11, wherein the IL-12p40 polypeptide comprises SEQ ID NO: 59.~~
- ~~13. The masked cytokine of claim 11, wherein the IL-12p40 polypeptide comprises SEQ ID NO: 60.~~
- ~~14. The masked cytokine of any one of claims 2-13, wherein the IL-12p35 polypeptide comprises SEQ ID NO: 2 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 2.~~
- ~~15. The masked cytokine of claim 14, wherein the IL-12p35 polypeptide comprises SEQ ID NO: 2.~~
- ~~16. The masked cytokine of any one of claims 1 to 7, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 4.~~
- ~~17. The masked cytokine of any one of claims 1 to 10, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 61.~~
- ~~18. The masked cytokine of any one of claims 1 to 10, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 62.~~
- ~~19. The masked cytokine of any one of claims 1 to 12, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 63.~~

- ~~20. The masked cytokine of any one of claims 1 to 13, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 64.~~
- ~~21. The masked cytokine of any one of the preceding claims, wherein the masking moiety comprises an IL-12 cytokine receptor, or a subunit or functional fragment thereof.~~
- ~~22. The masked cytokine of any one of the preceding claims, wherein the masking moiety comprises the extracellular domain of human IL-12R β 1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.~~
- ~~23. The masked cytokine of claim 22, wherein the masking moiety comprises residues 24 to 237 of human IL-12R β 1, namely a sequence having SEQ ID NO: 5.~~
- ~~24. The masked cytokine of claim 22, wherein the masking moiety comprises residues 24 to 545 of human IL-12R β 1, namely a sequence having SEQ ID NO: 6.~~
- ~~25. The masked cytokine of any one of claims 1-21, wherein the masking moiety comprises the extracellular domain of human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.~~
- ~~26. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 212 of human IL-12R β 2, namely a sequence having SEQ ID NO: 7.~~
- ~~27. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 222 of human IL-12R β 2, namely a sequence having SEQ ID NO: 8 or wherein the masking moiety comprises residues 24 to 227 of human IL-12R β 2, namely a sequence having SEQ ID NO: 11.~~
- ~~28. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9.~~
- ~~29. The masked cytokine of claim 28, wherein the masking moiety comprises at least one amino acid modification as compared to the sequence of SEQ ID NO: 9, optionally wherein said modifications are cysteine substitution mutations.~~

- ~~30. The masked cytokine of claim 29, wherein the masking moiety comprises SEQ ID NO: 65~~
- ~~31. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely a sequence having SEQ ID NO: 10.~~
- ~~32. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide is from 6 to 10 amino acids in length.~~
- ~~33. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 15.~~
- ~~34. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 41.~~
- ~~35. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 42.~~
- ~~36. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 43.~~
- ~~37. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 44.~~
- ~~38. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 45.~~
- ~~39. The masked cytokine of any one of the preceding claims, wherein the first polypeptide chain comprises:~~
- ~~N' **HL1-non-cleavable L1-MM** C'~~
- ~~and the second polypeptide chain comprises~~
- ~~N' **HL2-cleavable L2-C** C'~~
- ~~40. The masked cytokine of claim 39, wherein the non-cleavable linker is between 3 and 18 amino acids in length.~~

- ~~41. The masked cytokine of claim 39, wherein the non-cleavable linker is between 3 and 15 amino acids in length.~~
- ~~42. The masked cytokine of any one of claims 30 to 41, wherein the non-cleavable linker is rich in amino acid residues G and S.~~
- ~~43. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker includes $\{(G)_nS\}$, where $n=4$ or 5 .~~
- ~~44. The masked cytokine of any one of claims 39 to 43, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12 (GGGGS).~~
- ~~45. The masked cytokine of any one of claims 39 to 43, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13 (GGGGS GGGGS).~~
- ~~46. The masked cytokine of any one of claims 39 to 43, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14 (GGS GGGSGGGGS).~~
- ~~47. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 54 (GGS GGS GGS GGS GGS GP).~~
- ~~48. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 55 (PGGSGP).~~
- ~~49. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 56 (GGSPG).~~
- ~~50. The masked cytokine of any one of claims 39 to 49, wherein the cleavable linker comprises a proteolytically cleavable peptide (CP) flanked on both sides by a spacer domain (SD):~~
- ~~SD1-CP-SD2~~
- ~~where SD1 and SD2 are different, such that the first polypeptide chain comprises:~~
- ~~N'-HL1-non-cleavable-L1-MM-C'~~
- ~~and the second polypeptide chain comprises:~~
- ~~N'-HL2-SD1-CP-SD2-C-C'~~

51. The masked cytokine of claim 50, wherein the first spacer domain (SD1) is between 3 and 10 amino acids in length.
52. The masked cytokine of claims 50 or 51, wherein SD1 comprises SEQ ID NO: 16. (GGSGGS)
53. The masked cytokine of claims 50 or 51, wherein SD1 comprises SEQ ID NO: 17. (GGSGGSGGS)
54. The masked cytokine of any one of claims 50 to 53, wherein the second spacer domain (SD2) is between 3 and 6 amino acids in length.
55. The masked cytokine of any one of claims 50 to 54, wherein SD2 comprises SEQ ID NO: 18. (SGP)
56. The masked IL-2 cytokine of claim 50, wherein the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 44 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.
57. The masked IL-2 cytokine of claim 50, wherein the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 45 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.
58. The masked cytokine of any one of claims 50 to 54, wherein the cleavable linker comprises SEQ ID NO: 19. (GGSGGS**VPLSLY**SGP)
59. The masked cytokine of any one of claims 50 to 54, wherein the cleavable linker comprises SEQ ID NO: 20. (GGSGGSGGS**VPLSLY**SGP)
60. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 46. (GGSGGSM**PYDLYHP**SGP)
61. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 47. (GGSGGSGGS**MPYDLYHP**SGP)

- ~~62. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 48.
(GSGGSDSGGFMLTSGP)~~
- ~~63. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 49.
(GSGGSDSGGFMLTSGP)~~
- ~~64. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 50.
(GSGGSRAAAVKSPSGP)~~
- ~~65. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 51.
(GSGGSGSRRAAAVKSPSGP)~~
- ~~66. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 52.
(GSGGSISSGLLSGRSSGP)~~
- ~~67. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 53.
(GSGGSGSISSGLLSGRSSGP)~~
- ~~68. The masked cytokine of any one of the preceding claims, wherein the first half life extension domain comprises a first IgG1-Fc domain or a fragment thereof and the second half life extension domain comprises a second IgG1-Fc domain or a fragment thereof.~~
- ~~69. The masked cytokine of any one of the preceding claims, wherein the first and/or second Fc domains each contain one or more modifications that promote the non-covalent association of the first and the second half life extension domains.~~
- ~~70. The masked cytokine of any one of the preceding claims, wherein the first half life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).~~
- ~~71. The masked cytokine of any one of the preceding claims, wherein the first half life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).~~

~~72.5.~~_____ The masked cytokine of claim 1, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO:34 and a second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 40.

~~73.6.~~_____ A cleavage product capable of binding to IL-12R, the cleavage product comprising an IL-12 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in a masked IL-12 cytokine as defined in any of claims 1-~~42~~5.

~~74. A cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising a polypeptide comprising:~~

~~PCP-SD2-C~~

~~wherein PCP is a portion of a proteolytically cleavable peptide; SD2 is a spacer domain; and C is an IL-12 cytokine or functional fragment thereof.~~

~~75. The cleavage product of claim 74, wherein PCP is a portion of a proteolytically cleavable peptide as defined in any of claims 32 to 38.~~

~~76. The cleavage product of claims 74 or 75, wherein SD2 is a spacer domain as defined in any of claims 54 to 55.~~

~~77. The cleavage product of any one of claims 74 to 76, wherein C is an IL-12 cytokine or functional fragment thereof as defined in any of claims 2-20.~~

~~78. The cleavage product of any one of claims 74 to 77, wherein the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 29.~~

~~79. The cleavage product of claim 78, wherein the cleavage product comprises an amino acid sequence of SEQ ID NO: 29.~~

~~80.7.~~_____ A nucleic acid encoding the masked IL-12 cytokine of any one of claims 1-~~72~~5 or encoding one of the polypeptide chains of a masked IL-12 cytokine of any one of claims 1 to

~~725.~~

~~81.8.~~ A vector comprising the nucleic acid of claim ~~807~~.

~~82.9.~~ A host cell comprising nucleic acid encoding the masked IL-12 cytokine of any one of claims ~~1-51 to 72~~.

~~83.10.~~ A composition comprising the masked IL-12 cytokine of any one of claims ~~1-51-72~~.

~~84.11.~~ A pharmaceutical composition comprising the masked IL-12 cytokine of any one of claims ~~1-72~~ 1-5 and a pharmaceutically acceptable carrier.

~~85. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is in single unit dosage form.~~

~~86. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is formulated for intravenous administration and is in single unit dosage form.~~

~~87. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is formulated for injection and is in single unit dosage form.~~

~~88. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is a liquid and is in single unit dosage form.~~

~~89. A kit comprising the masked IL-12 cytokine of any one of claims 1-41, or the composition of claim 83, or the pharmaceutical composition of claims 84 to 88.~~

~~90.12.~~ A method of producing a masked IL-12 cytokine as defined in any one of claims 1 to ~~725~~ comprising culturing the host cell of claim ~~982~~ under a condition that produces the masked IL-12 cytokine.

~~91. A nucleic acid encoding the cleavage product of any one of claims 73 to 79.~~

~~92. A composition comprising the cleavage product of any one of claims 73 to 79.~~

~~93. A pharmaceutical composition comprising the cleavage product of any one of claims 73 to 79, and a pharmaceutically acceptable carrier.~~

~~94.13. Use of aA masked IL-12 cytokine as defined in any one of claims 1 to 572 for in the manufacture of a medicament for treating canceruse in medicine.~~

~~95. A cleavage product as defined in any one of claims 73 to 79 for use in medicine.~~

~~96. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine according to any one of claims 1 to 72.~~

~~97. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a composition according to claims 83 or 92.~~

~~98. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition according to any one of claims 84 to 88 and 93.~~

~~99. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine as defined in any one of claims 1 to 72, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in any one of claims 73 to 79.~~

~~100. A method of treating or preventing cancer in a subject, the method comprising a step of producing a cleavage product *in vivo* that is capable of binding to its cognate receptor, where the cleavage product is as defined in any one of claims 73 to 79.~~

~~101.14. The use A method according to any one of claims 96-100claim 13, wherein the cancer is a solid tumor.~~

~~102. A masked IL-12 cytokine as defined in any one of claims 1 to 72 for use in treating or preventing cancer.~~

~~103. A masked IL-12 cytokine as defined in any one of claims 1 to 72 for use in a method of treating or~~

~~preventing cancer, the method comprising administering to the subject an effective amount of the masked IL-12 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in any one of claims 73 to 79.~~

~~104. A masked IL-12 cytokine for use according to claim 103, wherein the cancer is a solid tumor.~~

~~105. A cleavage product as defined in any one of claims 73 to 79 for use in treating or preventing cancer.~~

~~106. A cleavage product as defined in any one of claims 73 to 79 for use in a method of treating or preventing cancer, the method comprising a step of administering a masked cytokine as defined in any one of claims 1 to 72 to a patient, thereby producing the cleavage product by proteolytic cleavage of the masked cytokine *in vivo*.~~

~~107. A cleavage product as defined in any one of claims 73 to 79 for use in a method of treating or preventing cancer in a subject, the method comprising a step of producing the cleavage product by *in vivo* proteolytic cleavage from a masked cytokine as defined in any one of claims 1 to 72 that has been administered to the subject.~~

~~108. A cleavage product for use according to any one of claims 105 to 107, wherein the cancer is a solid tumor.~~

~~109. A pharmaceutical composition according to any one of claims 84 to 88 and 93, for use in treating or preventing cancer.~~

~~110. A pharmaceutical composition for use according to claim 109, wherein the cancer is a solid tumor.~~