

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-2 cytokines, cleavage products of said masked IL-2 cytokines, and compositions thereof and methods of use thereof for addressing this need.

SUMMARY

The disclosed ~~invention~~disclosure relates to IL-2 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-2 cytokine or functional fragment thereof. The IL-2 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-2 cytokine or functional fragment thereof to its cognate receptor. Upon proteolytic cleavage of the cleavable linker at the target site, the IL-2 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-2 cytokine comprising a protein heterodimer comprising:

- a) a first polypeptide chain comprising a masking moiety linked to a first half-life extension domain via a first linker; and
- b) a second polypeptide chain comprising an IL-2 cytokine or functional fragment thereof linked to a second half-life extension domain via a second linker,

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 is a proteolytically cleavable linker comprising a proteolytically cleavable peptide.

In some ~~embodiment~~examples, the first half-life extension domain comprises a first Fc domain or a fragment thereof and the second Fc domain comprises an Fc domain or a fragment thereof.

In some ~~embodiment~~examples, the first Fc domain comprises a CH3 domain or a fragment thereof and the second Fc domain comprises a CH3 domain or a fragment thereof.

In some ~~embodiment~~examples, the first and second half-life extension domains are each an IgG1 Fc domain or 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.

In some [embodimentexamples](#), the first half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations Y349C; T366S; L38A; and Y407V to form a 'hole' in the first half-life extension domain and the second half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations S354C and T366W to form the 'knob' in the second half-life extension domain, numbered according to the Kabat EU numbering system.

In some [embodimentexamples](#), the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

In some [embodimentexamples](#), the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.

In some [embodimentexamples](#), the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

In some [embodimentexamples](#), the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

In some [embodimentexamples](#), the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some [embodimentexamples](#), the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some [embodimentexamples](#), the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some [embodimentexamples](#), the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodimentexamples, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

In some embodimentexamples, the masking moiety comprises IL-2R β or a fragment, portion or variant thereof.

In some embodimentexamples, the IL-2R β or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 4.

In some embodimentexamples, wherein the IL-2R β or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 5.

In some embodimentexamples, the second linker comprises a proteolytically cleavable peptide such that the second linker is a proteolytically cleavable linker and the first linker does not comprise a proteolytically cleavable peptide such that the first linker is a non- proteolytically cleavable linker.

In some embodimentexamples, the first linker comprises a proteolytically cleavable peptide such that the first linker is a proteolytically cleavable linker and the second linker does not comprise a proteolytically cleavable peptide such that the second linker is a non- proteolytically cleavable linker.

In some embodimentexamples, the proteolytically cleavable linker is from 10 to 25 amino acids in length.

In some embodimentexamples, the cleavable peptide within the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27 and 28.

In some embodimentexamples, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.

In some embodimentexamples, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.

In some embodimentexamples, the proteolytically cleavable linker comprises a proteolytically cleavable peptide flanked on both sides by a spacer domain.

In some embodimentexamples, the spacer domains are rich in amino acid residues G, S and P.

In some embodimentexamples, the spacer domains only include amino acid residue types selected from the group consisting of G, S and P.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 19, 20, 21 and 22.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17.

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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

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: 119 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

In some [embodimentexamples](#), the SD2 is from 3 to 6 amino acids in length.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 117.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.

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

In some [embodimentexamples](#), the non-proteolytically cleavable linker is between 3 and 8 amino acids in length.

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

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

In some [embodimentexamples](#), the non-proteolytically cleavable linker comprises an amino acid sequence of SEQ ID NO: 23.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 39.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 39 and a second polypeptide chain of SEQ ID NO: 49.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 40 and a second polypeptide chain of SEQ ID NO: 51.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 128.

In some [embodimentexamples](#), the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 129.

In some embodiment examples, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 130.

In some embodiment examples, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125 and a second polypeptide chain of SEQ ID NO: 51.

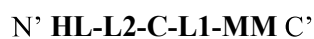
In some embodiment examples, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126 and a second polypeptide chain of SEQ ID NO: 51.

In some embodiment examples, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127 and a second polypeptide chain of SEQ ID NO: 51.

In another aspect, the present disclosure provides herein ~~is~~ a masked IL-2 cytokine comprising a masking moiety and an IL-2 cytokine or functional fragment thereof, wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor, and wherein a proteolytically cleavable peptide is present between the IL-2 fragment or functional fragment thereof and masking moiety.

In some embodiment examples, the masking moiety and IL-2 cytokine or functional fragment thereof are linked in a single polypeptide chain.

In some embodiment examples, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 1:



(1)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

In some embodiment examples, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 2:



(2)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

5 In some embodiment examples, the masking moiety comprises IL-2R β or a fragment, portion or variant thereof.

In some embodiment examples, the IL-2R β or a fragment, portion or variant thereof has mutations at amino acid positions C122 and C168 as compared to IL-2 β of SEQ ID NO: 4.

10 In some embodiment examples, the IL-2R β or a fragment, portion or variant thereof has mutations C122S and C168S as compared to IL-2 β of SEQ ID NO: 4.

15 In some embodiment examples, the half life extension domain (HL) comprises first and second half-life extension domains which are each an IgG1 Fc domain or fragment thereof.

In some embodiment examples, 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.

20 In some embodiment examples, the first half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations Y349C; T366S; L38A; and Y407V to form a 'hole' in the first half-life extension domain and the second half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations S354C and T366W to form the 'knob' in the second half-life extension domain, numbered according to the Kabat EU numbering system.

25 In some embodiment examples, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

30 In some embodiment examples, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.

35 In some embodiment examples, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

In some [embodimentexamples](#), the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

5 In some [embodimentexamples](#), the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.

In some [embodimentexamples](#), the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.

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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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

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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: 119 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

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In some [embodimentexamples](#), the SD2 is from 3 to 6 amino acids in length.

In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.

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In some [embodimentexample](#)s, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116.

In some [embodimentexample](#)s, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.

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In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.

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In some [embodimentexamples](#), the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.

~~In yet another aspect, the present disclosure provides~~ herein ~~is~~ a cleavage product capable of binding to its cognate receptor, the cleavage product comprising an IL-2 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in the masked IL-2 cytokine as defined in of any one of the statements or ~~embodiment examples~~ described herein.

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Provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a polypeptide comprising formula 3:

PCP-SD-C

(3)

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wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-2 cytokine or functional fragment thereof.

In some ~~embodiment examples~~, the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 polypeptide having SEQ ID NO: 2.

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In some ~~embodiment examples~~, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

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In some ~~embodiment examples~~, the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some ~~embodiment examples~~, the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A.

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In some ~~embodiment examples~~, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

In some ~~embodiment examples~~, the spacer domain is rich in amino acid residues G, S and P.

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In some ~~embodiment examples~~, the spacer domain only includes amino acid residue types selected from the group consisting of G, S and P.

In some ~~embodiment examples~~, the spacer domain comprises an amino acid sequence of any one of SEQ ID NO: 29, 30 and 31.

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In some ~~embodiment examples~~, the portion of the proteolytically cleavable peptide is a portion of an amino acid

sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.

In some embodiment examples, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.

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In some embodiment examples, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.

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In some embodiment examples, the cleavage product comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiment examples, the cleavage product comprises the amino acid sequence of SEQ ID NO: 137.

15 Provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a protein heterodimer comprising: a first polypeptide chain comprising a polypeptide comprising formula 4:

$$\text{HL1-SD-PCP}$$

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$$(4)$$

wherein HL1 is a first half-life extension domain; SD is a spacer domain; and PCP is a portion of a proteolytically cleavable peptide; and

25 a second polypeptide chain comprising a polypeptide comprising formula 5:

$$\text{HL2-L2-C}$$

$$(5)$$

30 wherein HL2 is a second half-life extension domain; L2 is a linker; and C is an IL-2 cytokine or functional fragment thereof; and

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

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In some embodiment examples, the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiment examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodimentexamples, the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodimentexamples, the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A.

In some embodimentexamples, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

In some embodimentexamples, the first half-life extension domain comprises a first Fc domain or a fragment thereof and the second Fc domain comprises an Fc domain or a fragment thereof.

In some embodimentexamples, the first Fc domain comprises a CH3 domain or a fragment thereof and the second Fc domain comprises a CH3 domain or a fragment thereof.

In some embodimentexamples, the first and second half-life extension domains are each an IgG1 Fc domain or 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.

In some embodimentexamples, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

In some embodimentexamples, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitutions N297A and I253A, numbered according to the Kabat EU numbering system.

In some embodimentexamples, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

In some embodimentexamples, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

In some embodiment examples, the second linker comprises an amino acid sequence of SEQ ID NO: 23.

In some embodiment examples, the spacer domain is rich in amino acids residues G, S and P.

- 5 In some embodiment examples, the spacer domain only includes amino acid residue types selected from the group consisting of G, S and P.

In some embodiment examples, the spacer domain comprises an amino acid sequence of SEQ ID NOs: 32, 33, 34, 35, 36 and 37.

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In some embodiment examples, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.

- 15 In some embodiment examples, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.

In some embodiment examples, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.

- 20 In some embodiment examples, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 136 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 135.

- 25 In some embodiment examples, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 138.

In some embodiment examples, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 141 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 140.

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In some embodiment examples, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 143 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 142.

- 35 In another aspect, the present disclosure provides herein ~~is~~ a nucleic acid encoding any one of the masked IL-2 cytokines described herein.

In another aspect, the present disclosure provides herein ~~is~~ a nucleic acid encoding one of the chains of any one of the masked IL-2 cytokines described herein.

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

Provided herein is a vector comprising a nucleic acid encoding a masked IL-2 cytokine described herein.

Provided herein is a vector comprising a nucleic acid encoding one of the chains of a masked IL-2 cytokine described herein.

In another aspect, the present disclosure provides a host cell comprising the nucleic acid encoding the masked IL-2 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 a further aspect, the present disclosure provides herein ~~is~~ a composition comprising any one of the masked IL-2 cytokines described herein.

In a further aspect, the present disclosure provides herein ~~is~~ a pharmaceutical composition comprising any one of the masked IL-2 cytokines described herein, and a pharmaceutically acceptable carrier.

Provided herein is a kit comprising any one of the masked IL-2 cytokines, or the compositions, or the pharmaceutical compositions described herein.

In a further aspect, the present disclosure provides herein ~~is~~ a method of producing any one of the masked IL-2 cytokines described herein, comprising culturing a host cell described herein under a condition that produces the masked IL-2 cytokine.

In a further aspect, the present disclosure provides a use of a masked IL-2 cytokine as defined herein in the manufacture of a medicament for treating cancer.

Provided herein is a nucleic acid encoding any one of the cleavage products described herein.

Provided herein is a composition comprising any one of the cleavage products described herein.

14a

Provided herein is a pharmaceutical composition comprising any one of the cleavage products described herein, and a pharmaceutically acceptable carrier.

Provided herein is a masked IL-2 cytokine as described herein for use in medicine.

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Provided herein is a cleavage product as 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-2 cytokine as 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 composition as 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 pharmaceutical composition as 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-2 cytokine as described herein, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as described herein.

15 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, where the cleavage product is as described herein.

Provided herein is a masked IL-2 cytokine as described herein for use in treating or preventing cancer.

20 Provided herein is a masked IL-2 cytokine as 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-2 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as described herein.

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

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

30 Provided herein is a cleavage product as 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 as described herein that has been administered to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

35 **FIG. 1** shows the structure of exemplary embodiments of a masked cytokine that includes a masking

CLAIMS

What is claimed is:

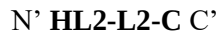
1. A masked IL-2 cytokine comprising a protein heterodimer comprising:
 - a) a first polypeptide chain comprising a masking moiety linked to a first half-life extension domain via a first linker; and
 - b) a second polypeptide chain comprising an IL-2 cytokine or functional fragment thereof linked to a second half-life extension domain via a second linker,
 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 is a proteolytically cleavable linker comprising a proteolytically cleavable peptide.

2. The masked IL-2 cytokine of claim 1, wherein the first polypeptide chain comprises formula 6:



(6)

and the second polypeptide chain comprises formula 5:



(5)

where HL1 is the first half life extension domain, L1 is the first linker, MM is the masking moiety, HL2 is the second half life extension domain, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof.

3. The masked IL-2 cytokine of claim 1 or claim 2, wherein the first half-life extension domain comprises a first Fc domain or a fragment thereof and the second half-life extension domain comprises an Fc domain or a fragment thereof.

~~4. The masked IL-2 cytokine of claim 3, 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.~~

~~5. The masked IL-2 cytokine of claim 3 or claim 4, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof.~~

- ~~6. The masked IL-2 cytokine of claims 5, wherein the first half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations Y349C; T366S; L38A; and Y407V to form a 'hole' in the first half-life extension domain and the second half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations S354C and T366W to form the 'knob' in the second half-life extension domain, numbered according to the Kabat EU numbering system.~~
- ~~7. The masked IL-2 cytokine of claims 5 or 6, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.~~
- ~~8. The masked IL-2 cytokine of any one of claims 5-7, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.~~
- 9.4. The masked IL-2 cytokine of claim 1 or claim 2, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.
- ~~10. The masked IL-2 cytokine of claim 1 or claim 2, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.~~
- ~~11. The masked IL-2 cytokine of any one of claims 1 to 10, wherein the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.~~
- 12.5. The masked IL-2 cytokine of any one of preceding claims ~~11~~, wherein IL-2 cytokine is thea modified IL-2 cytokine or functional fragment thereof ~~comprising~~comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.
- ~~13. The masked IL-2 cytokine of claim 11 or claim 12, wherein the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.~~
- ~~14. The masked IL-2 cytokine of any one of claims 11 to 13, wherein the modified IL-2 cytokine or~~

functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

~~15. The masked IL-2 cytokine of any one of claims 1 to 14, wherein the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.~~

~~16.6.~~ The masked IL-2 cytokine of any one of claims 1 to ~~155~~, wherein the masking moiety comprises IL-2R β or a fragment, portion or variant thereof.

~~17. The masked IL-2 cytokine of claim 16, wherein the IL-2R β or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 4.~~

~~18. The masked IL-2 cytokine of claim 16, wherein the IL-2R β or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 5.~~

~~19. The masked IL-2 cytokine of any one of claims 1 to 18, wherein the second linker comprises a proteolytically cleavable peptide such that the second linker is a proteolytically cleavable linker and the first linker does not comprise a proteolytically cleavable peptide such that the first linker is a non-proteolytically cleavable linker.~~

~~20. The masked IL-2 cytokine of any one of claims 1 to 18, wherein the first linker comprises a proteolytically cleavable peptide such that the first linker is a proteolytically cleavable linker and the second linker does not comprise a proteolytically cleavable peptide such that the second linker is a non-proteolytically cleavable linker.~~

~~21. The masked IL-2 cytokine of claim 19 or claim 20, wherein the non-proteolytically cleavable linker is between 3 and 18 amino acids in length.~~

~~22. The masked IL-2 cytokine of claim 21, wherein the non-proteolytically cleavable linker is between 3 and 8 amino acids in length.~~

~~23. The masked IL-2 cytokine of any one of claims 19 to 22, wherein the non-proteolytically cleavable linker is rich in amino acid residues G, S and P.~~

- ~~24. The masked IL-2 cytokine of any one of claims 19 to 23, wherein the non-proteolytically cleavable linker comprises an amino acid sequence of SEQ ID NO: 14.~~
- ~~25. The masked IL-2 cytokine of any one of claims 19 to 23, wherein the non-proteolytically cleavable linker comprises an amino acid sequence of SEQ ID NO: 23.~~
- ~~26. The masked IL-2 cytokine of any one of claims 1 to 25, wherein the proteolytically cleavable linker is from 10 to 25 amino acids in length.~~
- ~~27. The masked IL-2 cytokine of any one of claims 1 to 26, wherein the cleavable peptide within the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27 and 28.~~
- ~~28. The masked IL-2 cytokine of any one of claims 1 to 26, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.~~
- ~~29. The masked IL-2 cytokine of any one of claims 1 to 26, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.~~
- ~~30. The masked IL-2 cytokine of any one of claims 1 to 29, wherein the proteolytically cleavable linker comprises a proteolytically cleavable peptide flanked on both sides by a spacer domain.~~
- ~~31. The masked IL-2 cytokine of claim 30, wherein the spacer domains are rich in amino acid residues G, S and P.~~
- ~~32. The masked IL-2 cytokine of claim 30 or claim 31 wherein the spacer domains only include amino acid residue types selected from the group consisting of G, S and P.~~
- ~~33. The masked IL-2 cytokine of any one of claims 1 to 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 19, 20, 21 and 22.~~
- ~~34. The masked IL-2 cytokine of claim 33, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19.~~

- ~~35. The masked IL-2 cytokine of claim 33, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17.~~
- ~~36. The masked IL-2 cytokine of claim 30, 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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.~~
- ~~37. The masked IL-2 cytokine of claim 30, 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: 119 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.~~
- ~~38. The masked IL-2 cytokine of claim 36 or claim 37, wherein SD2 is from 3 to 6 amino acids in length.~~
- ~~39. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.~~
- ~~40. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116 and 117.~~
- ~~41. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.~~
- ~~42. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.~~
- ~~43. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.~~
- ~~44. The masked IL-2 cytokine of claim 1 or claim 19, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38.~~
- ~~45. The masked IL-2 cytokine of claim 1 or claim 19, wherein the masked IL-2 cytokine comprises a first~~

~~polypeptide chain of SEQ ID NO: 39.~~

~~46. The masked IL-2 cytokine of claim 1 or claim 20, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125.~~

~~47. The masked IL-2 cytokine of claim 1 or claim 20, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126.~~

~~48. The masked IL-2 cytokine of claim 1 or claim 20, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127.~~

~~49. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 39 and a second polypeptide chain of SEQ ID NO: 49.~~

~~50. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 40 and a second polypeptide chain of SEQ ID NO: 51.~~

~~51. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 128.~~

~~52. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 129.~~

~~53. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 130.~~

~~54. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125 and a second polypeptide chain of SEQ ID NO: 51.~~

~~55. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126 and a second polypeptide chain of SEQ ID NO: 51.~~

~~56. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127 and a second polypeptide chain of SEQ ID NO: 51.~~

~~57.7.~~ A masked IL-2 cytokine comprising a masking moiety and an IL-2 cytokine or functional fragment thereof, wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor, and wherein a proteolytically cleavable peptide is present between the IL-2 fragment or functional fragment thereof and masking moiety.

~~58. A masked IL-2 cytokine according to claim 57, wherein the masking moiety and IL-2 cytokine or functional fragment thereof are linked in a single polypeptide chain.~~

~~59. A masked IL-2 cytokine according to claim 57 or claim 58, wherein the masked IL-2 cytokine comprises a polypeptide chain comprising formula 1:~~



(1)

~~where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.~~

~~60. A masked IL-2 cytokine according to claim 57 or claim 58, wherein the masked IL-2 cytokine comprises a polypeptide chain comprising formula 2:~~



(2)

~~where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.~~

~~61. The masked IL-2 cytokine of any one of claims 57 to 60, wherein the masking moiety comprises IL-2R β or a fragment, portion or variant thereof.~~

~~62. The masked cytokine of claim 61, wherein the IL-2R β or a fragment, portion or variant thereof has mutations at amino acid positions C122 and C168 as compared to IL-2 β of SEQ ID NO: 4.~~

~~63. The masked cytokine of claim 61 or claim 62, wherein the IL-2R β or a fragment, portion or variant thereof has mutations C122S and C168S as compared to IL-2 β of SEQ ID NO: 4.~~

- ~~64. The masked IL-2 cytokine of any one of claims 57 to 63, wherein the half life extension domain (HL) comprises first and second half life extension domains which are each an IgG1 Fc domain or fragment thereof.~~
- ~~65. The masked IL-2 cytokine of claim 64, 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.~~
- ~~66. The masked IL-2 cytokine of claim 64 or claim 65, wherein the first half life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations Y349C; T366S; L38A; and Y407V to form a 'hole' in the first half life extension domain and the second half life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations S354C and T366W to form the 'knob' in the second half life extension domain, numbered according to the Kabat EU numbering system.~~
- ~~67. The masked IL-2 cytokine of any one of claims 64 to 66, wherein the first and second half life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.~~
- ~~68. The masked IL-2 cytokine of any one of claims 64 to 67, the first and second half life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.~~
- ~~69. The masked IL-2 cytokine of any one of claims 64 to 67, wherein the first half life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.~~
- ~~70. The masked IL-2 cytokine of any one of claims 64 to 68, wherein the first half life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.~~
- ~~71. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.~~

- ~~72. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.~~
- ~~73. The masked IL-2 cytokine of claim 71, 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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.~~
- ~~74. The masked IL-2 cytokine of claim 72, 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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.~~
- ~~75. The masked IL-2 cytokine of claim 73 or claim 74, wherein SD2 is from 3 to 6 amino acids in length.~~
- ~~76. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.~~
- ~~77. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116.~~
- ~~78. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.~~
- ~~79. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.~~
- ~~80. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.~~

~~81-8.~~ 807. A cleavage product capable of binding to its cognate receptor, the cleavage product comprising an IL-2 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in the masked IL-2 cytokine as defined in of any one of claims 1 to ~~807~~.

~~82. A cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a polypeptide comprising formula 3:~~



~~(3)~~

~~wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-2 cytokine or functional fragment thereof.~~

~~83. The cleavage product of claim 81 or claim 82, wherein the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 polypeptide having SEQ ID NO: 2.~~

~~84. The cleavage product of claim 83, wherein the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.~~

~~85. The cleavage product of claim 83 or claim 84, wherein the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.~~

~~86. The cleavage product of any one of claims 83 to 85, wherein the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.~~

~~87. The cleavage product of any one of claims 83 to 86, wherein the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.~~

~~88. The cleavage product of any one of claims 82 to 87, wherein the spacer domain is rich in amino acid residues G, S and P.~~

~~89. The cleavage product of any one of claims 82 to 88, wherein the spacer domain only includes amino acid residue types selected from the group consisting of G, S and P.~~

~~90. The cleavage product of any one of claims 82 to 89, wherein the spacer domain comprises an amino acid sequence of any one of SEQ ID NO: 29, 30 and 31.~~

~~91. The cleavage product of any one of claims 82 to 90, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.~~

~~92. The cleavage product of any one of claims 82 to 90, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.~~

~~93. The cleavage product of any one of claims 82 to 90, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.~~

~~94. The cleavage product of claim 82, comprising the amino acid sequence of SEQ ID NO: 56.~~

~~95. The cleavage product of claim 82, comprising the amino acid sequence of SEQ ID NO: 137.~~

~~96. A cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a protein heterodimer comprising:~~

~~a) a first polypeptide chain comprising a polypeptide comprising formula 4:~~

~~HL1-SD-PCP~~

~~(4)~~

~~wherein HL1 is a first half life extension domain; SD is a spacer domain; and PCP is a portion of a proteolytically cleavable peptide; and~~

~~b) a second polypeptide chain comprising a polypeptide comprising formula 5:~~

~~HL2-L2-C~~

~~(5)~~

~~wherein HL2 is a second half life extension domain; L2 is a linker; and C is an IL-2 cytokine or functional fragment thereof; and~~

~~wherein the first half life extension domain is associated with the second half life extension domain.~~

~~97. The cleavage product of claim 96, wherein the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.~~

~~98. The cleavage product of claim 97, wherein the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.~~

- ~~99. The cleavage product of claim 97 or claim 98, wherein the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.~~
- ~~100. The cleavage product of any one of claims 97 to 99, wherein the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A.~~
- ~~101. The cleavage product of any one of claims 97 to 100, wherein the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.~~
- ~~102. The cleavage product of any one of claims 96 to 101, wherein the first half-life extension domain comprises a first Fc domain or a fragment thereof and the second Fc domain comprises an Fc domain or a fragment thereof.~~
- ~~103. The cleavage product of claim 102, wherein the first Fc domain comprises a CH3 domain or a fragment thereof and the second Fc domain comprises a CH3 domain or a fragment thereof.~~
- ~~104. The cleavage product of claim 102 or claim 103, 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.~~
- ~~105. The cleavage product of any one of claims 96 to 104, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof.~~
- ~~106. The cleavage product of claim 105, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.~~
- ~~107. The cleavage product of claim 105 or claim 106, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitutions N297A and I253A, numbered according to the Kabat EU numbering system.~~
- ~~108. The cleavage product of claim 105, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises~~

~~the amino acid sequence of SEQ ID NO: 12.~~

~~109.—The cleavage product of claim 105, wherein the first half life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.~~

~~110.—The cleavage product of any one of claims 96 to 109, wherein the second linker comprises an amino acid sequence of SEQ ID NO: 23.~~

~~111.—The cleavage product of any one of claims 96 to 110, wherein the spacer domain is rich in amino acids residues G, S and P.~~

~~112.—The cleavage product of any one of claims 96 to 111, wherein the spacer domain only includes amino acid residue types selected from the group consisting of G, S and P.~~

~~113.—The cleavage product of any one of claims 96 to 112, wherein the spacer domain comprises an amino acid sequence of SEQ ID NOs: 32, 33, 34, 35, 36 and 37.~~

~~114.—The cleavage product of any one of claims 96 to 113, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.~~

~~115.—The cleavage product of any one of claims 96 to 113, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.~~

~~116.—The cleavage product of any one of claims 96 to 113, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.~~

~~117.—The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 59-B and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 59-A.~~

~~118.—The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain having an amino acid sequence of~~

~~SEQ ID NO: 138.~~

~~119.—The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 141 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 140.~~

~~120.—The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 143 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 142.~~

~~121.9.~~ A nucleic acid encoding the masked IL-2 cytokine of any one of claims 1 to ~~807~~ or encoding one of the polypeptide chains of a masked IL-2 cytokine of any one of claims 1 to ~~807~~.

~~122.10.~~ A vector comprising the nucleic acid of claim ~~1219~~.

~~123.11.~~ A host cell comprising the nucleic acid encoding the masked IL-2 cytokine of any one of claims ~~1 to 809-10~~.

~~124.12.~~ A composition comprising the masked IL-2 cytokine of any one of claims ~~1 to 801-7~~.

~~125.13.~~ A pharmaceutical composition comprising the masked IL-2 cytokine of any one of claims 1 to ~~807~~, and a pharmaceutically acceptable carrier.

~~126.—A kit comprising the masked IL-2 cytokine of any one of claims 1 to 80, or the composition of claim 124, or the pharmaceutical composition of claim 125.~~

~~127.14.~~ A method of producing a masked IL-2 cytokine as defined in any one of claims 1 to ~~780~~ comprising culturing the host cell of claim ~~123-11~~ under a condition that produces the masked IL-2 cytokine.

~~128.—A nucleic acid encoding the cleavage product of any one of claims 81 to 120.~~

~~129.—A composition comprising the cleavage product of any one of claims 81 to 120.~~

~~130.—A pharmaceutical composition comprising the cleavage product of any one of claims 81 to 120, and a pharmaceutically acceptable carrier.~~

~~131.—The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is in single unit dosage form.~~

~~132.—The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is formulated for intravenous administration and is in single unit dosage form.~~

~~133.—The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is formulated for injection and is in single unit dosage form.~~

~~134.—The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is a liquid and is in single unit dosage form.~~

~~135.15. Use of aA masked IL-2 cytokine as defined in any one of claims 1 to 80 for in the manufacture of a medicament for treating canceruse in medicine.~~

~~136.—A cleavage product as defined in any one of claims 81 to 120 for use in medicine.~~

~~137.—A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-2 cytokine according to any one of claims 1 to 80.~~

~~138.—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 claim 124 or claim 129.~~

~~139.—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 125 and 130 to 134.~~

~~140.—A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-2 cytokine as defined in any one of claims 1 to 80, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in one of claims 81 to 120.~~

- ~~141.— 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 81 to 120.~~
- ~~142.— A method according to any one of claims 137 to 141, wherein the cancer is a solid tumor.~~
- ~~143.— A masked IL-2 cytokine as defined in any one of claims 1 to 80 for use in treating or preventing cancer.~~
- ~~144.— A masked IL-2 cytokine as defined in any one of claims 1 to 80 for use in a method of treating or preventing cancer, the method comprising administering to the subject an effective amount of the masked IL-2 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in one of claims 81 to 120.~~
- ~~145.— A masked IL-2 cytokine for use according to claim 143 or claim 144, wherein the cancer is a solid tumor.~~
- ~~146.— A cleavage product as defined in any one of claims 81 to 120 for use in treating or preventing cancer.~~
- ~~147.— A cleavage product as defined in any one of claims 81 to 120 for use in treating or preventing cancer, the method comprising a step of administering a masked cytokine as defined in any one of claims 1 to 80 to a patient, thereby producing the cleavage product by proteolytic cleavage of the masked cytokine *in vivo*.~~
- ~~148.— A cleavage product as defined in any one of claims 81 to 120 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 80 that has been administered to the subject.~~
- ~~149.— A cleavage product for use according to any one of claims 143 to 148, wherein the cancer is a solid tumor.~~