

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 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 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 comprising an IL-12p40 polypeptide covalently linked to an IL-12p35 polypeptide via an IL-12p40 – IL-12p35 linker,

wherein the first half-life extension domain is associated with the second half-life extension domain, wherein the first linker is a non-cleavable linker, and

wherein the second linker comprises a cleavable peptide of SEQ ID NO: 44 (ISSGLLSGRS), SEQ ID NO: 41 (MPYDLYHP), or SEQ ID NO: 43 (RAAAVKSP).

In some examples, 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.

Provided 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 examples 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 examples, PCP is a portion of a proteolytically cleavable peptide as described herein.

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

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

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

In another aspect, the present disclosure provides herein a nucleic acid encoding any one of the masked IL-12 cytokines described herein.

Provided 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 provides herein 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.

8a

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 the nucleic acid as described
5 herein. Provided herein is a host cell comprising a nucleic acid described herein.

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

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

5

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

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

10

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

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

15

In some 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.

20

In another aspect, the present disclosure provides herein 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.

25

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

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

30 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 or preventing cancer.

35

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

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

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 comprising an IL-12p40 polypeptide covalently linked to an IL-12p35 polypeptide via an IL-12p40 – IL-12p35 linker,

wherein the first half-life extension domain is associated with the second half-life extension domain, wherein the first linker is a non-cleavable linker,

and

wherein the second linker comprises a cleavable peptide of SEQ ID NO: 44 (ISSGLLSGRS), SEQ ID NO: 41 (MPYDLYHP), or SEQ ID NO: 43 (RAAAVKSP).

2. The masked IL-12 cytokine of claim 1, wherein the IL-12p40 polypeptide comprises an amino acid sequence of SEQ ID NO: 60.
3. The masked IL-12 cytokine of claim 1, wherein the IL-12p35 polypeptide comprises an amino acid sequence of SEQ ID NO: 2.
4. The masked IL-12 cytokine of claim 1, wherein the first linker is between 3 to 18 amino acids in length.
5. The masked IL-12 cytokine of claim 1 or claim 4, wherein the first linker is rich in amino acid residues G and S.
6. The masked IL-12 cytokine of claim 1, wherein the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length.

7. The masked IL-12 cytokine of claim 1 or claim 6, wherein the IL-12p40 – IL-12p35 linker is rich in amino acid residues G and S.
8. The masked IL-12 cytokine of any one of claims 1 to 7, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 64.
9. The masked IL-12 cytokine of claim 1, 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.
10. The masked IL-12 cytokine of claim 1 or claim 9, wherein the masking moiety comprises SEQ ID NO: 65.
11. The masked IL-12 cytokine of claim 1, 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.
12. The masked IL-12 cytokine of claim 1 or claim 11, 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.
13. The masked IL-12 cytokine of any one of claims 1, 11, or 12, wherein the first half-life extension domain comprises SEQ ID NO: 25 and the second half-life extension domain comprises SEQ ID NO: 26.
14. A nucleic acid encoding the masked IL-12 cytokine of any one of claims 1-13.
15. A vector comprising the nucleic acid of claim 14.
16. A host cell comprising the nucleic acid of claim 14.
17. A composition comprising the masked IL-12 cytokine of any one of claims 1-13.
18. A pharmaceutical composition comprising the masked IL-12 cytokine of any one of claims 1-13 and a pharmaceutically acceptable carrier.

19. A method of producing a masked IL-12 cytokine as defined in any one of claims 1-13 comprising culturing the host cell of claim 16 under a condition that produces the masked IL-12 cytokine.
20. Use of a masked IL-12 cytokine as defined in any one of claims 1-13 in the manufacture of a medicament for treating or preventing cancer.
21. The use according to claim 20, wherein the cancer is a solid tumor.