leakage, through systemic immune activation. These findings illustrate the need for developing therapeutics, such as cytokine therapeutics, that effectively target tumors without the side effects associated with systemic immune activation.

Prodrugs in which a cytokine therapeutic is masked by a masking moiety and in which the therapeutic is only active after cleavage of the masking moiety in the tumor cell environment are one way envisaged for addressing this need.

SUMMARY

This disclosure provides novel tumor-specific proteolytically cleavable peptide linkers comprising tumor-specific proteolytically cleavable peptides and their use in polypeptide drug constructs for delivering a therapeutic moiety to a tumor cell environment. The part of the construct other than the therapeutic moiety can be considered as a carrier moiety.

The tumor-specific proteolytically cleavable peptide acts as a substrate for protease(s) present in the tumor cell environment. The proteolytically cleavable peptide linker is positioned within the polypeptide drug construct so that the linker cleaves by protease action in the tumor cell environment, and the polypeptide drug construct separates to form cleavage products, one of which will comprise the therapeutic moiety. This disclosure also relates to cleavage products of said drug constructs, and methods related to the use of the same.

In one aspect, provided herein is a polypeptide drug construct comprising (i) a therapeutic moiety; (ii) a carrier moiety and (iii) a proteolytically cleavable peptide linker comprising a tumor-specific proteolytically cleavable peptide having an amino acid sequence DLLAVVAAS or ISSGLLSGRS.

In a second aspect, the present disclosure provides a masked cytokine comprising:

a) a first polypeptide chain comprising a masking moiety linked to a first half-life extension moiety via a first linker; and

b) a second polypeptide chain comprising a cytokine moiety thereof linked to a second half-life extension moiety via a second linker,

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

wherein at least the first linker or the second linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

In some examples, the proteolytically cleavable peptide (CP) is flanked on both sides by a spacer domain

(SD1 and SD2) as shown in formula:

SD1-CP-SD2.

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

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

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

In some examples, the first spacer domain (SD1) is between 3 and 6 amino acids in length.

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

In some examples, SD2 comprises the amino acid sequence SGP.

In some examples, SD2 has the amino acid sequence SGP.

In some examples, the proteolytically cleavable peptide linker comprises sequence GGPS**DLLAVVAAS**SGP.

In some examples, the proteolytically cleavable peptide linker comprises sequence GSGPS**DLLAVVAAS**SGP.

In some examples, the proteolytically cleavable peptide linker comprises sequence GSSGGP**DLLAVVAAS**SGP.

In some examples, the proteolytically cleavable peptide linker comprises sequence GSP**DLLAVVAAS**SGP.

In some examples, the proteolytically cleavable peptide linker comprises sequence GSPG**DLLAVVAAS**SGP.

In some examples, the proteolytically cleavable peptide linker comprises sequence GSGSPS**DLLAVVAAS**SGP.

In some examples, the proteolytically cleavable linker comprises sequence GGSSGGSP**ISSGLLSGRS**SGPGSGS.

In some examples, the proteolytically cleavable linker comprises sequence GPPSGSSP**ISSGLLSGRS**SGGG.

In some examples, the proteolytically cleavable linker comprises sequence GGSGGS**ISSGLLSGRS**SGP.

In some examples, the proteolytically cleavable linker comprises sequence GGSGGSGGS**ISSGLLSGRS**SGP.

In some examples, the proteolytically proteolytically cleavable peptide linker is covalently bonded directly to the therapeutic moiety.

In some examples, the proteolytically cleavable peptide linker is located within the drug construct between the therapeutic moiety and the carrier moiety.

In some examples, the proteolytically cleavable peptide linker is located within the carrier moiety.

In some examples, the polypeptide drug construct comprises a single polypeptide chain. This means that the therapeutic moiety, the carrier moiety and the proteolytically cleavable peptide linker are present in the same polypeptide chain.

In some examples, the polypeptide drug construct comprises more than one polypeptide chain. In some examples, the proteolytically cleavable peptide linker is present in the same polypeptide chain as the therapeutic moiety. In some examples, the proteolytically cleavable peptide linker is present in a different polypeptide chain to the therapeutic moiety.

In some examples, the polypeptide drug construct is a prodrug. In some examples, where the polypeptide drug construct is a prodrug, the remainder of the molecule (away from which the therapeutic moiety separates after cleavage of the proteolytically cleavable peptide linker) comprises a masking moiety, which inhibits the biological activity of the therapeutic moiety in the prodrug such that the therapeutic moiety is biologically active only after cleavage of the proteolytically cleavable peptide linker in the tumor cell environment In some examples, the masking moiety is present in the same polypeptide chain as the therapeutic moiety. In some examples, the masking moiety is present in a different polypeptide chain to the therapeutic moiety.

In some examples, the masking moiety is present in the same polypeptide chain as the therapeutic moiety.

In some examples, the masking moiety is present in a first polypeptide chain and the therapeutic moiety is present in a second polypeptide chain.

In some examples, the drug construct comprises a half-life extension moiety.

In some examples, the half-life extension moiety comprises an antibody or fragment thereof.

In some examples, the half-life extension moiety comprises first and second half-life extension moieties.

In some examples, the prodrug is a cytokine prodrug where the therapeutic moiety is a cytokine moiety.

In some examples, the masking moiety comprises a domain of the extracellular domain of the cytokine receptor.

A cytokine prodrug as described herein, where the therapeutic moiety is a cytokine moiety and the masking moiety comprises a domain of the extracellular domain of the cytokine receptor is referred to herein as a "masked cytokine".

Provided herein, in some examples, is a masked cytokine comprising a masking moiety in a first polypeptide chain and a cytokine moiety thereof in a second polypeptide chain. Such masked cytokines may be referred to as 'heterodimeric' masked cytokines.

In some examples, the masked cytokine comprises a protein heterodimer comprising:

a) a first polypeptide chain comprising a masking moiety linked to a first half-life extension moiety via a first linker; and
b) a second polypeptide chain comprising a cytokine moiety thereof linked to a second half-life extension moiety via a second linker,

wherein the first half-life extension moiety is associated with the second half-life extension moiety, and wherein at least the first linker or the second linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

In some examples, in the first polypeptide chain, the first half life extension domain is linked to the amino terminus of the first linker and the carboxy terminus of the first linker is linked to the amino terminus of the masking moiety and, in the second polypeptide chain, the second half life extension domain is linked to the amino terminus of the second linker and the carboxy terminus of the second linker is linked to the amino terminus of the cytokine moiety thereof.

In some examples, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

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

wherein at least the first linker or the second linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

In some examples, the second linker is the proteolytically cleavable linker and the first linker is a non-cleavable linker. This arrangement is described herein as 'Structure A'.

In some examples, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

In some examples, the first linker is the proteolytically cleavable linker and the second is a non-cleavable linker. This arrangement is described herein as 'Structure B'.

In some examples, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

Provided herein, in some examples, is a masked cytokine comprising a masking moiety and a cytokine moiety thereof linked in a single polypeptide chain. In another aspect, the present disclosure provides a masked cytokine comprising a polypeptide chain comprising formula:

N' **HL-L2-C-L1-MM** C'

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 cytokine moiety, wherein at least the first linker comprises a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS. The proteolytically cleavable peptide linker may be as described anywhere herein. In some examples, the first linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS. In some examples, the first linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence ISSGLLSGRS. In some examples, the first linker is a proteolytically cleavable peptide linker and the second linker is non-cleavable. The non-cleavable linker may be as described anywhere herein.

In another aspect, the present disclosure provides a masked cytokine comprising a polypeptide chain comprising formula:

N' **HL-L2-MM-L1-C** C'

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 cytokine moiety thereof, wherein at least the first linker comprises a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS. The proteolytically cleavable peptide linker may be as described anywhere herein. In some examples, the first linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS. In some examples, the first linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence ISSGLLSGRS. In some examples, the first linker is a proteolytically cleavable peptide linker and the second linker is non-cleavable. The non-cleavable linker may be as described anywhere herein.

In some examples, the non-cleavable linker is between 3 and 25 amino acids in length.

In some examples, wherein the non-cleavable linker is rich in amino acid residues G, S and P.

In some examples, the non-cleavable linker comprises an amino acid sequence of SEQ ID NO: 14.

In some examples, the non-cleavable linker comprises an amino acid sequence of SEQ ID NO: 23.

In some examples, the half-life extension domain comprises a first half life extension domain and a second half life extension domain.

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

In some 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.

In some 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.

In some examples, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise an amino substitution at position 297, numbered according to the Kabat EU numbering system.

In some 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.

In some examples, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise an amino substitution at position 253, numbered according to the Kabat EU numbering system.

In some 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.

In some 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 examples, 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 examples, the half life extension domain (HL) comprises an Fc region of an antibody (i.e. the C-terminal region of an immunoglobulin heavy chain) or a fragment thereof comprising dimerized Fc domains (HL1-HL2). Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. In some examples, the dimerized Fc domains of an antibody (HL1-HL2) comprises a first half life extension domain and a second half life extension domain as described anywhere herein, where the first half-life extension moiety comprises a first Fc domain or a fragment thereof and the second half-life extension moiety comprises a second Fc domain or a fragment thereof. In some examples, HL2 is a component of the polypeptide chain and HL1 is dimerized to HL2.

In some examples, the first and second half-life extension moieties are each an IgG1 Fc domain or fragment thereof. In some examples, the first half-life extension moiety comprises an IgG1 Fc domain or fragment thereof including the mutation I253A and the second half-life extension moiety comprises an IgG1 Fc domain or fragment thereof including the mutation I253A. In some examples, the first and second half-life extension moieties are derived from the sequence for human IgG1 Immunoglobulin heavy constant gamma 1 having SEQ ID NO: 6 (the 'parent sequence'), such that the first and second half-life extension moieties each comprise SEQ ID NO: 7 or fragment thereof, with one or more amino acid modifications. In some examples, the first and second half-life extension moieties comprise SEQ ID NO: 7 with amino substitutions to promote association of the first and second half-life extension moieties according to the 'knob into holes' approach. In some examples, the sequence SEQ ID NO: 7 contains mutations Y349C; T366S; L38A; and Y407V (numbered according to the Kabat EU numbering system) to form the 'hole' in the first half-life extension moiety and mutations S354C and T366W (numbered according to the Kabat EU numbering system) to form the 'knob' in the second half-life extension moiety. In some examples, the first and second half-life extension moieties each further comprise amino substitution N297A, numbered according to the Kabat EU numbering system. In some examples, the first and second half-life extension moieties each further comprise the amino substitution I253A, numbered according to the Kabat EU numbering system. In some examples, the first and second half-life extension moieties each further comprise both the amino substitutions N297A and I253A, numbered according to the Kabat EU numbering system. In some examples, the first half-life extension moiety 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 any one of the amino acid sequence of any one of SEQ ID NOs: 7, 8, 9 and 10. In some examples, the second half-life extension moiety 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 any one of the amino acid sequence of any one of SEQ ID NOs: 7, 11, 12 and 13.
In some examples, the cytokine moiety comprises a wild-type cytokine moiety or variant cytokine moiety.

In some examples, the cytokine moiety is an IL-2 cytokine moiety as described anywhere herein.

In some examples, the IL-2 cytokine moiety comprises a wild-type IL-2 cytokine moiety or variant thereof.

In some examples, the IL-2 cytokine moiety comprises an IL-2 cytokine or fragment thereof.

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

In some examples, the masking moiety comprises IL-2Rβ or a fragment, portion or variant thereof.

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

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

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

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

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

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

In some examples, the cytokine moiety is an IL-12 cytokine moiety as described anywhere herein.

In some examples, the IL-12 cytokine moiety comprises a wild-type IL-12 cytokine moiety or variant thereof.

In some examples, the IL-12 cytokine moiety comprises an IL-12 cytokine or fragment thereof.

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

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

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

In some examples, the IL-12p40 – IL-12p35 linker comprises SEQ ID NO: 116 (GGGGSGGGGSGGGGS).

In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 204 (as shown in the IL-12 Cytokine Moieties table in the description) or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 204 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 204 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, 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: 204 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 205 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 206 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, 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: 204 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 207 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 208 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12p35 polypeptide comprises SEQ ID NO: 209 (as shown in the IL-12 Cytokine Moieties table in the description) or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 209 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12p35 polypeptide comprises SEQ ID NO: 209 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 210 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 211 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 212 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 213 (as shown in the IL-12 Cytokine Moieties table in the description).

In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 214 (as shown in the IL-12 Cytokine Moieties table in the description).

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

In some examples, the masking moiety comprises the extracellular domain of human IL-12Rβ1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

In some examples, the masking moiety comprises residues 24 to 237 of human IL-12Rβ1, namely a sequence having SEQ ID NO: 215 (as shown in the IL-12 Masking Moieties table in the description).

In some examples, the masking moiety comprises residues 24 to 545 of human IL-12Rβ1, namely a sequence having SEQ ID NO: 216 (as shown in the IL-12 Masking Moieties table in the description).

In some examples, the masking moiety comprises the extracellular domain of human IL-12Rβ2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

In some examples, the masking moiety comprises residues 24 to 212 of human IL-12Rβ2, namely a sequence having SEQ ID NO: 217 (as shown in the IL-12 Masking Moieties table in the description).

In some examples, the masking moiety comprises residues 24 to 222 of human IL-12Rβ2, namely a sequence having SEQ ID NO: 218 (as shown in the IL-12 Masking Moieties table in the description), or the masking moiety comprises residues 24 to 227 of human IL-12Rβ2, namely a sequence having SEQ ID NO: 222 (as shown in the IL-12 Masking Moieties table in the description).

In some examples, the masking moiety comprises residues 24 to 319 of human IL-12Rβ2, namely a sequence having SEQ ID NO: 219 (as shown in the IL-12 Masking Moieties table in the description).

In some examples, the masking moiety comprises at least one amino acid modification as compared to the sequence of SEQ ID NO: 219 (as shown in the IL-12 Masking Moieties table in the description), optionally wherein said modifications are cysteine substitution mutations.

In some examples, the masking moiety comprises SEQ ID NO: 220 (as shown in the IL-12 Masking Moieties table in the description).

In some examples, the masking moiety comprises residues 24 to 622 of human IL-12Rβ2, namely a sequence having SEQ ID NO: 221 (as shown in the IL-12 Masking Moieties table in the description).

In some examples, the cytokine moiety is an IL-15 cytokine moiety as described anywhere herein.

In some examples, the IL-15 cytokine moiety comprises a wild-type IL-15 cytokine moiety or variant thereof.

In some examples, the cytokine moiety is an IL-15 cytokine moiety and the masked cytokine further comprises a domain comprising an IL-15Rα subunit or a functional fragment thereof ('IL-15Rα domain'). In some examples, the cytokine moiety is an IL-15 cytokine moiety and the masked cytokine further comprises a domain comprising an IL-15Rα subunit or a functional fragment thereof ('IL-15Rα domain'), and the IL-15Rα domain and the IL-15 cytokine moiety are present in different polypeptide chains in the construct and the IL-15Rα domain is non-covalently linked to the IL-15 cytokine moiety.

The 'IL-15Rα domain' herein can consist of the sequence of the wild-type sushi domain sIL-15Rα or a variant thereof, such as the sequence of the wild-type sushi domain sIL-15Rα with one or more e.g. 1, 2, 3

or 4 amino acid substitutions. In some examples, the IL-15Rα domain comprises an amino acid substitution at position R26. In some examples, the IL-15Rα domain comprises amino acid substitution R26N. In some examples, the IL-15Rα domain comprises amino acid substitution R26S. In some examples, the IL-15Rα domain comprises an amino acid substitution at position R35. In some examples, the IL-15Rα domain comprises amino acid substitution R35Q. In some examples, the IL-15Rα domain comprises amino acid substitution R35S. In some examples, the IL-15Rα domain comprises an amino acid substitution at positions R26 and R35. In some examples, the IL-15Rα domain comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some examples, the IL-15Rα domain comprises amino acid substitutions R26N and R35Q.

In some examples, the IL-15 cytokine moiety comprises an IL-15 cytokine or fragment thereof.

In some examples, the IL-15 cytokine or fragment thereof comprises SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description) or a functional fragment thereof.

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53 as compared to the amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53, N71, N79, or N112 as compared to the amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N79 as compared to the amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N112 as compared to the amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71, N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 224 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 225 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 226 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 227 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 228 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 229 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 230 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 233 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 234 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 235 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 236 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 237 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 238 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 239 (as shown in the IL-15 Cytokine Moieties table in the description).

In some examples, the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position N71.

In some examples, the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position S73.

In some examples, the IL-15 cytokine or functional fragment thereof comprises an additional mutation at one or more of amino acid positions N72, N79, V80, T81, and N112.

In some examples, the masking moiety comprises IL-15Rβ or a fragment or variant thereof.

In some examples, the masking moiety comprises the amino acid sequence of SEQ ID NO: 240 (as shown in the IL-15 Masking Moieties table in the description).

In some examples, the masking moiety comprises IL-15Rβ variant or a fragment thereof having an amino acid substitution at position C122.

In some examples, the masking moiety comprises IL-15Rβ variant or a fragment thereof having amino acid substitution C122S.

In some examples, the masking moiety comprises IL-15Rβ variant or a fragment thereof having an amino acid substitution at position C168.

In some examples, the masking moiety comprises IL-15Rβ variant or a fragment thereof having amino acid substitution C168S.

In some examples, the masking moiety comprises IL-15Rβ variant or a fragment thereof having an amino acid substitution at positions C122 and C168.

In yet another aspect, provided herein is a cleavage product comprising an active therapeutic moiety, preparable by proteolytic cleavage of the proteolytically cleavable linker in the polypeptide drug constructs as described anywhere herein.

Provided herein is a nucleic acid encoding any one of the polypeptide drug constructs as described anywhere herein described herein.

Provided herein is a nucleic acid encoding one of the chains of any one of the polypeptide drug constructs as described anywhere herein described herein.

Provided herein is a vector comprising a nucleic acid described herein.

Provided herein is a vector comprising a nucleic acid encoding a polypeptide drug construct as described anywhere herein described herein.

Provided herein is a vector comprising a nucleic acid encoding one of the chains of a polypeptide drug constructs as described anywhere herein described herein.

In another aspect, the present disclosure provides a host cell comprising a nucleic acid encoding a masked cytokine as described 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.

Provided herein is a composition comprising any one of the polypeptide drug constructs as described anywhere herein described herein.

Provided herein is a pharmaceutical composition comprising any one of the polypeptide drug constructs as described anywhere herein described herein, and a pharmaceutically acceptable carrier.

Provided herein is a kit comprising any one of the polypeptide drug constructs as described anywhere herein, or the compositions, or the pharmaceutical compositions described herein.

Provided herein is a method of producing any one of the polypeptide drug constructs as described anywhere herein, comprising culturing a host cell described herein under a condition that produces the polypeptide drug construct.

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.

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

Provided herein is a polypeptide drug construct as described herein for use in medicine.

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

In another aspect, the present disclosure provides a use of a polypeptide drug construct as described herein in the manufacture of a medicament for treating cancer in a subject. 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 polypeptide drug construct 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 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.

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 polypeptide drug construct as described herein, whereby the polypeptide drug construct is proteolytically cleaved *in vivo* to produce a cleavage product as 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 target protein, where the cleavage product is as described herein.

Provided herein is a polypeptide drug construct as described herein for use in treating or preventing cancer.

Provided herein is a polypeptide drug construct 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 polypeptide drug construct, whereby the polypeptide drug construct is proteolytically cleaved *in vivo* to produce a cleavage product as described herein.

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 polypeptide drug construct as described herein to a patient,

CLAIMS

1. A polypeptide drug construct comprising (i) a therapeutic moiety; (ii) a carrier moiety and (iii) a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

2. A polypeptide drug construct according to claim 1, wherein the proteolytically cleavable peptide (CP) is flanked on both sides by a spacer domain (SD1 and SD2) as shown in formula:

SD1-CP-SD2.

3. A polypeptide drug construct according to claim 2, wherein the first spacer domain (SD1) and/or second spacer domain (SD2) is between 3 and 6 amino acids in length.

4. A polypeptide drug construct according to claim 1, wherein the proteolytically cleavable peptide linker is covalently bonded directly to the therapeutic moiety.

5. A polypeptide drug construct according to claim 1, wherein the polypeptide drug construct comprises more than one polypeptide chain.

6. A polypeptide drug construct according to claim 1, further comprising a masking moiety.

7. A polypeptide drug construct according to claim 1, wherein the polypeptide drug construct comprises a half-life extension moiety.

8. A masked cytokine comprising:

a) a first polypeptide chain comprising a masking moiety linked to a first half-life extension moiety via a first linker; and

b) a second polypeptide chain comprising a cytokine moiety thereof linked to a second half-life extension moiety via a second linker,

wherein the first half-life extension moiety is associated with the second half-life

extension moiety, and

wherein at least the first linker or the second linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

9. A masked cytokine according to claim 8, wherein the first polypeptide chain comprises:

N' HL1-L1-MM C'

and the second polypeptide chain comprises:

N' HL2-L2-C C'

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 cytokine moiety,

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

wherein at least the first linker or the second linker is a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

10. A masked cytokine comprising a polypeptide chain comprising formula:

N' **HL-L2-C-L1-MM** C'

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 cytokine moiety,

wherein at least the first linker comprises a proteolytically cleavable peptide linker comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

11. A masked cytokine comprising a polypeptide chain comprising formula:

N' **HL-L2-MM-L1-C** C'

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 cytokine moiety thereof,

wherein at least the first linker comprises a proteolytically cleavable peptide linker

comprising a proteolytically cleavable peptide (CP) consisting of the amino acid sequence DLLAVVAAS or ISSGLLSGRS.

12. A masked cytokine according to claim 11, wherein the proteolytically cleavable peptide (CP) is flanked on both sides by a spacer domain (SD1 and SD2) as shown in formula:

SD1-CP-SD2.

13. A masked cytokine according to claim 11, wherein the second linker is a cleavable or non-cleavable linker.

14. A masked cytokine according to claim 11, wherein the half-life extension domain comprises a first half life extension domain and a second half life extension domain.

15. A masked cytokine according to claim 11, wherein the cytokine moiety is an IL-2 cytokine moiety.

16. A masked cytokine according to claim 11, wherein the cytokine moiety is an IL-12 cytokine moiety.

17. A masked cytokine according to claim 11, wherein the cytokine moiety is an IL-15 cytokine moiety.

18. A cleavage product comprising an active therapeutic moiety, preparable by proteolytic cleavage of the proteolytically cleavable linker in a polypeptide drug construct according to claim 1.

19. A host cell comprising a nucleic acid encoding a masked cytokine of claim 11.

20. Use of a polypeptide drug construct according to claim 1 in the manufacture of a medicament for treating cancer in a subject.