

creates significant CMC issues due to the heterogeneous products of random conjugation methods.

[0004] Accordingly, there remains a need for novel immunotherapy approaches to promote tumor-localization and increase efficacy, while preventing systemic toxicity.

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SUMMARY

[0005] Described herein are compounds, compositions, and methods for treating cancer. The compounds include a fusion protein including each of an IL-2, an IL-12, a collagen-binding domain, and a linear polypeptide spacer. When administered to a subject, the compounds have a favorable residence time in the tumor and can provide in some examples treatments with acceptable toxicity an enhanced therapeutic index. In some examples, the collagen-binding domain binds to the collagen in the tumor to maintain localization of the compound in the tumor for an extended period of time.

[0006] In one aspect, disclosed herein is an immunomodulatory fusion protein comprising: (i) an IL-2; (ii) an IL-12; (iii) a collagen-binding domain, wherein the collagen-binding domain comprises a leucine-rich repeat from a human proteoglycan Class II member of the small leucine-rich proteoglycan (SLRP) family; or a human type I glycoprotein having an Ig-like domain, and (iv) a linear polypeptide spacer.

[0006a] In another aspect, disclosed herein is a pharmaceutical composition comprising any one of the immunomodulatory fusion proteins disclosed herein and a pharmaceutically acceptable carrier.

[0006b] In another aspect, disclosed herein is the use of any one of the immunomodulatory fusion proteins disclosed herein in the manufacture of a pharmaceutical composition for activating, enhancing or promoting a response by an immune cell in a subject or inhibiting, reducing or suppressing a response by an immune cell in a subject, or treating cancer, or reducing or inhibiting tumor growth.

[0006c] In yet another aspect, disclosed herein is the use of any one of the immunomodulatory fusion proteins disclosed herein in the manufacture of a pharmaceutical composition for reducing or inhibiting tumor growth or treating cancer in a subject in combination with a second composition comprising (i) a tumor antigen-targeting antibody, (ii) a cancer vaccine, (iii) an immune checkpoint inhibitor, or (iv) an adoptive cell therapy, thereby reducing or inhibiting tumor growth or treating cancer in the subject.

[0006d] In yet another aspect, disclosed herein is an immunomodulatory fusion protein comprising:

(i) an IL-2;

(ii) an IL-12;

5 (iii) a LAIR2 collagen-binding domain,

wherein LAIR2 comprises at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 15; and

(iv) an albumin;

10 wherein the albumin comprises at least about 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 16-18.

[0007] In various examples, the immunomodulatory fusion protein is linear. In various examples, the immunomodulatory fusion protein is a continuous chain. In various examples, the immunomodulatory fusion protein is a continuous polypeptide chain.

15 **[0008]** In various examples, the IL-2 is at the N-terminus of the immunomodulatory fusion protein. In various examples, the IL-12 is at the C-terminus of the immunomodulatory fusion protein. In various examples, the IL-2 is at the N-terminus of the immunomodulatory fusion protein and the IL-12 is at the C-terminus of the immunomodulatory fusion protein.

20 **[0009]** In various examples, the linear polypeptide spacer is positioned in between the IL-2 and the collagen-binding domain. In various examples, the collagen-binding domain is positioned in between the IL-12 and the linear polypeptide spacer.

[0010] In various examples, the C-terminus of the IL-2 is operably linked to the N-terminus of the linear polypeptide spacer. In various examples, the C-terminus of the IL-2 is operably linked by a linker to the N-terminus of the linear polypeptide spacer.

25 **[0011]** In various examples, the C-terminus of the linear polypeptide spacer is operably linked to the N-terminus of the collagen-binding domain. In various examples, the C-terminus of the linear polypeptide spacer is operably linked by a linker to the N-terminus of the collagen-binding domain.

[0012] In various examples, the C-terminus of the collagen-binding domain is operably linked to the N-terminus of the the IL-12. In various examples, the C-terminus of the collagen-binding domain is operably linked by a linker to the N-terminus of the the IL-12.

[0013] In various examples, the collagen-binding domain is positioned in between the IL-2 and the linear polypeptide spacer. In various examples, the linear polypeptide spacer is positioned in between the IL-12 and the collagen-binding domain. In various examples, the C-terminus of the IL-2 is operably linked to the N-terminus of the collagen-binding domain.

[0014] In various examples, the C-terminus of the IL-2 is operably linked by a linker to the N-terminus of the collagen-binding domain. In various examples, the C-terminus of the collagen-binding domain is operably linked to the N-terminus of the linear polypeptide spacer.

[0015] In various examples, the C-terminus of the collagen-binding domain is operably linked by a linker to the N-terminus of the linear polypeptide spacer. In various examples, the C-terminus of the linear polypeptide spacer is operably linked to the N-terminus of the the IL-12. In various examples, the C-terminus of the linear polypeptide spacer is operably linked by a linker to the N-terminus of the the IL-12.

[0016] In various examples, the IL-2 is at the C-terminus of the immunomodulatory fusion protein. In various examples, the IL-12 is at the N-terminus of the immunomodulatory fusion protein. In various examples, the IL-2 is at the C-terminus and the IL-12 is at the N-terminus of the immunomodulatory fusion protein.

[0017] In various examples, the N-terminus of the IL-2 is operably linked to the C-terminus of the linear polypeptide spacer. In various examples, the N-terminus of the IL-2 is operably linked by a linker to the C-terminus of the linear polypeptide spacer.

[0018] In various examples, the N-terminus of the linear polypeptide spacer is operably linked to the C-terminus of the collagen-binding domain. In various examples, the N-terminus of the linear polypeptide spacer is operably linked by a linker to the C-terminus of the collagen-binding domain.

[0019] In various examples, the N-terminus of the collagen-binding domain is operably linked to the C-terminus of the the IL-12. In various examples, the N-terminus of the collagen-binding domain is operably linked by a linker to the C-terminus of the the IL-12.

[0020] In various examples, the collagen-binding domain is positioned in between the IL-2 and the linear polypeptide spacer. In various examples, the linear polypeptide spacer is positioned in between the IL-12 and the collagen-binding domain.

[0021] In various examples, the N-terminus of the IL-2 is operably linked to the C-terminus of the collagen-binding domain. In various examples, the N-terminus of the IL-2 is operably linked by a linker to the C-terminus of the collagen-binding domain.

[0022] In various examples, the N-terminus of the collagen-binding domain is operably linked to the C-terminus of the linear polypeptide spacer. In various examples, the N-terminus of the collagen-binding domain is operably linked by a linker to the C-terminus of the linear polypeptide spacer.

[0023] In various examples, the N-terminus of the linear polypeptide spacer is operably linked to the C-terminus of the IL-12. In various examples, the N-terminus of the linear polypeptide spacer is operably linked by a linker to the C-terminus of the IL-12.

[0024] In various examples, one or more of the linkers are the same. In various examples, one or more of the linkers are the different.

[0025] In various examples, the IL-12 is at the C terminus of the immulatory fusion protein and is operably linked to the collagen binding domain, which is operably linked to a linear polypeptide spacer, which is operably linked to the IL-2 at the N terminus of the protein, and wherein the protein is linear.

[0026] In various examples, the IL-12 is at the N terminus of the immulatory fusion protein and is operably linked to the collagen binding domain, which is operably linked to a linear polypeptide spacer, which is operably linked to the IL-2 at the C terminus of the protein, and wherein the protein is linear.

[0027] In various examples, the IL-12 is at the C terminus of the immulatory fusion protein and is operably linked to the linear polypeptide spacer, which is operably linked to collagen binding domain, which is operably linked to the IL-2 at the N terminus of the protein, and wherein the protein is linear.

[0028] In various examples, the IL-12 is at the N terminus of the immulatory fusion protein and is operably linked to the linear polypeptide spacer, which is operably linked to collagen binding domain, which is operably linked to the IL-2 at the C terminus of the protein, and wherein the protein is linear.

[0029] In various examples, the immulatory fusion protein further comprises a second linear polypeptide spacer.

[0030] In various examples, the IL-12 is at the N terminus of the immulatory fusion protein and is operably linked to the first linear polypeptide spacer, which is operably linked to the collagen binding domain, which is operably linked to the second linear polypeptide spacer,

which is operably linked to the IL-2 at the C terminus of the protein, and wherein the protein is linear.

[0031] In various examples, the IL-12 is at the C terminus of the immunomodulatory fusion protein and is operably linked to the first linear polypeptide spacer, which is operably linked to the

5 collagen binding domain, which is operably linked to the second linear polypeptide spacer, which is operably linked to the IL-2 at the N terminus of the protein, and wherein the protein is linear.

[0032] In various examples, the immunomodulatory fusion protein is a continuous chain. In various examples, the immunomodulatory fusion protein is a continuous polypeptide chain.

[0033] In various examples, the collagen-binding domain comprises (i) a leucine-rich repeat
10 from a human proteoglycan Class II member of the small leucine-rich proteoglycan (SLRP) family which comprises lumican; or (ii) a human type I glycoprotein having an Ig-like domain selected from LAIR1 and LAIR2.

[0034] In various examples, the collagen-binding domain comprises lumican. In various examples, the lumican comprises at least about 80% sequence identity to the amino acid sequence
15 as set forth in SEQ ID NO: 11.

[0035] In various examples, the collagen-binding domain comprises LAIR 1. In various examples, the LAIR1 comprises at least about 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 13. In various examples, the LAIR1 comprises at least 80% identity to the amino acid as set forth in SEQ ID NO: 14.

20 **[0036]** In various examples, the collagen-binding domain comprises LAIR 2. In various examples, the LAIR2 comprises at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 15.

[0037] In various examples, the IL-2 comprises human IL-2. In various examples, the IL-2 comprises human wild-type IL-2. In various examples, the IL-2 comprises at least about 80%
25 sequence identity to the amino acid sequence as set forth in SEQ ID NO: 1. In various examples, the IL-2 comprises at least about 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 2.

[0038] In various examples, the IL-12 comprises human IL-12. In various examples, the IL-12 comprises human wild-type IL-12. In various examples, the IL-12 comprises at least about 80%
30 sequence identity to the amino acid sequence as set forth in SEQ ID NO: 5. In various examples, the IL-12 comprises at least about 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 6.

[0039] In various examples, the linear polypeptide spacer is an albumin. In various examples, the linear polypeptide spacer is an albumin binding domain. In various examples, the albumin comprises human albumin.

5 **[0040]** In various examples, the albumin comprises at least about 80% sequence identity to the amino acid sequence as set forth in SEQ ID NOs: 16-18. In various examples, the albumin binding domain comprises at least about 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 19.

[0041] In various examples, the immunomodulatory fusion protein molecular weight is at least about 100-1000 kDa..

10 **[0042]** Additionally disclosed herein is an pharmaceutical composition comprising an immunomodulatory fusion protein of any one of the immunomodulatory fusion proteins disclosed herein, and a pharmaceutically acceptable carrier.

[0043] Additionally disclosed herein is a method for activating, enhancing or promoting a response by an immune cell in a subject or inhibiting, reducing or suppressing a response by an immune cell in a subject, comprising administering to a subject in need thereof, an effective
15 amount of the pharmaceutical composition of any one of the pharmaceutical composition disclosed herein.

[0044] Additionally disclosed herein is a method for treating cancer, or reducing or inhibiting tumor growth, comprising administering to a subject in need thereof, an effective amount of the pharmaceutical composition of any one of the pharmaceutical composition disclosed herein.
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[0045] In various examples, the subject has at least one tumor. In various examples, the composition is administered intratumorally (i.tu) or peritumorally (peri.tu) to the at least one tumor. In various examples, the at least one tumor size is reduced or substantially identical to a reference standard. In various examples, the reference standard is the size of the tumor prior to administration.
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[0046] In various examples, the composition is administered by injection.

[0047] In various examples, the composition has an intratumoral retention $t_{1/2}$ of more than 24 hours.

[0048] In various examples, twelve hours after intratumoral injection less than 25% of the injected dose is detected in the serum.
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[0049] In various examples, the at least one tumor has stromal CD8+ cytotoxic T cells (CTL) $\leq$ 50 cells/mm². In various examples, the at least one tumor has stromal CD8+ cytotoxic T cells (CTL) $\geq$ 50 cells/mm² and intraepithelial compartment CD8+ cytotoxic T cells

(CTL) $\leq$ 500 cells/mm². In various examples, the at least one tumor has intraepithelial compartment CD8+ cytotoxic T cells (CTL) $\geq$ 500 cells/mm².

[0050] In various examples, the method does not result in cytokine release syndrome in the subject. In various examples, the subject does not experience grade 4 cytokine release syndrome.

5 **[0051]** Additionally disclosed herein is a method for reducing or inhibiting tumor growth or treating cancer in a subject, the method comprising administering to a subject in need thereof, an effective amount of the pharmaceutical composition of any one of the pharmaceutical composition disclosed herein, and an effective amount of a second composition comprising (i) a tumor antigen-targeting antibody, (ii) a cancer vaccine, (iii) an immune checkpoint inhibitor, or
10 (iv) an adoptive cell therapy, thereby reducing or inhibiting tumor growth or treating cancer in the subject.

[0052] In various examples, the tumor antigen is a tumor-associated antigen (TAA), a tumor specific antigen (TSA), or a tumor neoantigen and/or wherein the tumor antigen-targeting antibody specifically binds human HER-2/neu, EGFR, VEGFR, CD20, CD33, CD38 or antigen-
15 binding fragment thereof. In various examples, the cancer vaccine is a peptide comprising one or more tumor-associated antigens, or a population of cells immunized in vitro with a tumor antigen and administered to the subject. In various examples, the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof which binds PD-1, PD-L1, CTLA-4, LAG3, or TIM3. In various examples, the immune effector cell comprises a chimeric antigen receptor
20 (CAR) molecule which binds to a tumor antigen.

In certain examples, described herein is an immunomodulatory fusion protein comprising: an IL-2; an IL-12; a LAIR2 collagen-binding domain, wherein LAIR2 comprises at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 15; and an albumin; wherein the albumin comprises at least about 80% sequence identity to the amino acid sequence as set forth in SEQ ID
25 NO: 16-18.

BRIEF DESCRIPTION OF THE DRAWINGS

[0053] **FIG. 1** illustrates exemplary bi-functional linear fusion collagen-localized immunomodulatory constructs comprising an IL-12, a collagen-binding domain, an albumin, and an IL-2.

30 **[0054]** **FIGs. 2A-2F** are graphs showing recombinant proteins purified with NiNTA resin and evaluated for product quality using analytical size exclusion chromatography (SEC). **FIG. 2A** shows the SEC profile of the 12-MSA-Lum-MSA-2 construct (HMW = 19%; Main = 79%; LMW = 2%). **FIG. 2B** shows the SEC profile of the 12-Lum-MSA-2 construct (HMW = 9%; Main =