

SUMMARY

[5] Provided herein are therapeutic proteins, nucleic acids (e.g., DNA, RNA, mRNA) that encode the proteins, and compositions and methods of using the proteins and nucleic acids for the treatment of a disease or disorder, such as proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, graft-versus-host disease and the like. In certain embodimentsexamples, the fusion protein the amino acid sequence of any one of SEQ ID NOs.: 193-271. Certain fusion proteins disclosed herein are referred to as ACP200-208, ACP211, ACP213-ACP215, ACP240-ACP245, ACP247, ACP284-ACP292, ACP296-ACP300, ACP302-ACP306, ACP309-ACP314, ACP336-ACP359, ACP371-ACP379, ACP383-ACP434, ACP439-ACP447, or ACP451-ACP471. This disclosure also relates to nucleic acids (e.g., DNA, RNA, mRNA) that encode the fusion proteins, methods of making fusion proteins, compositions comprising a fusion protein, as well as methods of using the fusion proteins to treat cancer including combination of two or more fusion proteins and one or more fusion protein in combination with another therapeutic agent.

[6] The invention-disclosure features fusion proteins that are conditionally active variants of a cytokine of interest. Cytokines of particular interest include IL-2, IL-12, and IFN. In one aspectexample, the full-length polypeptides of the inventiondisclosure have reduced or minimal cytokine-receptor activating activity even though they contain a functional cytokine polypeptide. Upon activation, e.g., by cleavage of a linker that joins a blocking moiety, e.g. a steric blocking polypeptide, in sequence to the active cytokine, the cytokine, e.g., IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, IFNalpha, IFNbeta, IFNgamma, TNFalpha, lymphotoxin, TGF-beta1, TGFbeta2, TGFbeta3, GM-CSF, CXCL10, CCL19, CCL20, CCL21 or functional fragment or mutein or functional variant or a subunit of any of the foregoing, can bind its receptor and effect signaling. If desired, the full-length polypeptides can include a blocking polypeptide moiety that also provides additional advantageous properties. For example, the full-length polypeptide can contain a blocking polypeptide moiety that also extends the serum half-life and/or targets the full-length polypeptide to a desired site of cytokine activity. Alternatively, the full-length fusion polypeptides can contain a serum half-life extension element and/or targeting domain that are distinct from the blocking polypeptide moiety. Preferably, the fusion protein contains at least one element or domain capable of extending *in vivo* circulating half-life. Preferably, this element is removed enzymatically in the desired body location (e.g. protease cleavage in the tumor microenvironment), restoring pharmacokinetic properties to the payload molecule (e.g. IL2, IL-12, IFNb or IFNa) substantially similar to the naturally occurring payload molecule. The fusion proteins may be targeted to a desired cell or tissue.

[7] The fusion polypeptides typically comprise a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H], and a protease-cleavable polypeptide linker. The cytokine polypeptide and the blocking moiety and the optional half-life extension element when

present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, e.g., the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker. Some preferred fusion polypeptides are of one of formula (I) – (VI):

[A]-[L1]-[H]-[L2]-[D] (I);

[D]-[L2]-[H]-[L1]-[A] (II);

[A]-[L1]-[D]-[L2]-[H] (III);

[H]-[L2]-[D]-[L1]-[A] (IV);

[H]-[L1]-[A]-[L2']-[D] (V);

[D]-[L1]-[A]-[L2']-[H] (VI);

wherein A is a cytokine polypeptide, D is a blocking moiety, H is a half-life extension moiety, L1 is a protease-cleavable polypeptide linker, L2 is an polypeptide linker that is optionally protease-cleavable, and L2' is a protease-cleavable polypeptide linker. L1 and L2 or L1 and L2' can be have the same or different amino acid sequence and or protease-cleavage site (when L2 is protease-cleavable) as desired.

[8] In some aspectsexamples, the fusion proteins described herein are conditionally active variants of IL-12, IL-2, or IFN. In embodimentsexamples, the fusion protein can contain an IL-2 polypeptide. The fusion protein containing an IL-2 polypeptide can comprise or consist of the amino acid sequence of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, and 636-646. The fusion proteins disclosed as SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646 are referred to herein as ACP289-ACP292, ACP296-ACP302, WW0301, ACP304-ACP306, ACP309-ACP313, WW0353, ACP414, ACP336-ACP398, WW0472-WW0477, ACP406-ACP426, ACP439-ACP447, ACP451-ACP471, WW0729, WW0734-WW0792, ACP101, ACP293-ACP295, ACP316-ACP335, ACP427-ACP438, and ACP448-ACP450. For example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 272. The fusion protein can comprise the amino acid sequence of SEQ ID NO: 286. The fusion protein can comprise the amino acid sequence of SEQ ID NO:362. The fusion protein can comprise the amino acid sequence of SEQ ID NO:336. The fusion protein can comprise the amino acid sequence of SEQ ID NO: 348. The fusion protein can comprise the amino acid sequence of SEQ ID NO: 363. The fusion protein can comprise the amino acid sequence of SEQ ID NO: 580. In one aspect, the disclosure relates to a fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, and 636-646 or an amino acid sequence that has at least about 80% identity to SEQ

ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, and 636-646.

[9] In embodimentsexamples, the fusion protein can contain IL-12. The fusion protein containing an IL-12 polypeptide can comprise or consist of the amino acid sequence of any one of SEQ ID NOs. 368-371, 434-440, 453-519, or 523-538. The fusion proteins disclosed as SEQ ID NOs. 368-371, 434-440, 453-519, or 523-538 are referred to herein as ACP240-ACP245, ACP247, ACP285-ACP288, WW0641, WW0649-WW0652, WW0662-WW0725, WW0765-WW0772, and WW0796-WW0803.

For

example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 424. For example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 428. For example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 541. For example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 556. For example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 560. For example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 568. For example, the fusion protein can comprise the amino acid sequence of SEQ ID NO: 573. In one aspect, the disclosure relates to a fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 368-371, 434-440, 453-519, and 523-538, or an amino acid sequence that has at least about 80% identity to SEQ ID NOs. 368-371, 434-440, 453-519, and 523-538.

[10] In ~~embodiments~~examples, the fusion protein contains IFN. The fusion protein containing an IFN polypeptide can comprise or consist of the amino acid sequence of any one of SEQ ID NOs. 421-430, and 539-578. The fusion proteins disclosed as SEQ ID NOs. 421-430, and 539-578 can be referred to herein as ACP200-ACP209, WW0644-WW0648, WW0781-WW0786, WW0815-WW0822, WW0831-WW0834, WW0737-WW0748, and WW0787-WW0790. In one aspect, the disclosure relates to a fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 421-430, and 539-578, or an amino acid sequence that has at least 80% identity to SEQ ID NOs. 421-430, and 539-578.

[11] In some examples~~aspects~~, the fusion polypeptide disclosed herein can be covalently or non-covalently bonded to a second polypeptide chain. For example, a fusion polypeptide can dimerize (i.e. form a dimer) or a portion of a fusion polypeptide may associate with another polypeptide, for example, to form a functional binding site for a cytokine polypeptide or serum albumin. In certain ~~embodiments~~examples, the second polypeptide chain and the blocking moiety on the fusion polypeptide are complementary and together form a functional binding site that has specificity for the cytokine polypeptide contained in the fusion polypeptide. Exemplary functional binding sites that can be formed by the blocking moiety of the fusion polypeptide and a complimentary second polypeptide include antigen binding sites of antibodies, such as a Fab fragment of an antibody or a portion thereof. For example, one chain of a Fab that binds the cytokine can be the blocking moiety of the fusion polypeptide, e.g., a VH-CH1, and the complementary VL-CL can be part of the second polypeptide. In such situations, the blocking moiety of the fusion protein i.e., VH-CH1 and the second polypeptide that comprises the complementary VL-CL, can associate to form a functional binding site with specificity for the cytokine polypeptide contained within the fusion protein (e.g., IL-2, IL-12, IFNalpha, IFNbeta) and attenuate cytokine polypeptide activity.

[12] In ~~embodiments~~examples, the fusion protein containing an IL-2 cytokine polypeptide can be bonded covalently or noncovalently to a second polypeptide chain. The second polypeptide chain can contain an antibody light chain VL-CL that comprises or consists of the amino acid sequence of SEQ

ID NO: 263, 264, or 333. Such a second polypeptide can bond with a complimentary VH-CH1 polypeptide contained within the fusion protein, e.g., as contained within SEQ ID NOS: 362, 363, 325, 286, 579, 581, or 582. The second polypeptide chain disclosed as SEQ ID NOs. 263, 264, and 333 can be referred herein as WW0523 (ACP381), WW0524 (ACP382), or WW0556 (ACP414). In one aspect, the disclosure relates to a polypeptide comprising a first polypeptide that is covalently or non-covalently bonded to a second polypeptide, wherein the first polypeptide comprises an amino acid sequence selected from SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 or an amino acid sequence that has at least 80% identity to the SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 and the second polypeptide sequence comprises an amino acid sequence of SEQ ID NOs: 263, 264, or 333 or an amino acid sequence that has at least 80% identity to SEQ ID NOs: 263, 264, or 333.

[13] In ~~embodiments~~ examples, the fusion polypeptide can comprise or consist of the amino acid sequence of SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 and the second polypeptide chain can comprise

sequence of SEQ ID NO: 233. For example, the fusion protein can comprise or consist of SEQ ID NO: 581 and the second polypeptide chain can comprise or consist of SEQ ID NO.: 263. For example, the fusion protein can comprise or consist of SEQ ID NO: 581 and the second polypeptide chain can comprise or consist of SEQ ID NO.: 264. For example, the fusion protein can comprise or consist of SEQ ID NO: 581 and the second polypeptide chain can comprise or consist of SEQ ID NO.: 333. For example, the fusion protein can comprise or consist of SEQ ID NO: 582 and the second polypeptide chain can comprise or consist of SEQ ID NO: 263. For example, the fusion protein can comprise or consist of SEQ ID NO: 582 and the second polypeptide chain can comprise or consist of SEQ ID NO: 264. For example, the fusion protein can comprise or consist of SEQ ID NO: 582 and the second polypeptide chain can comprise or consist of SEQ ID NO: 333.

[14] As described herein targeting is accomplished through the action of a blocking polypeptide moiety that also binds to a desired target, or through a targeting domain. The domain that recognizes a target antigen on a preferred target (for example a tumor-specific antigen), may be attached to the cytokine via a cleavable or non-cleavable linker. If attached by a non-cleavable linker, the targeting domain may further aid in retaining the cytokine in the tumor, and it may be considered a retention domain. The targeting domain does not necessarily need to be directly linked to the payload molecule, and it may be linked directly to another element of the fusion protein. This is especially true if the targeting domain is attached via a cleavable linker.

[15] In one ~~example~~aspect is provided a fusion polypeptide comprising a cytokine polypeptide, or functional fragment, a mutein thereof, a functional variant thereof or a subunit thereof, and a blocking moiety, e.g. a steric blocking domain. The blocking moiety is fused to the cytokine polypeptide, directly or through a linker, and can be separated from the cytokine polypeptide by cleavage (e.g., protease mediated cleavage) of the fusion polypeptide at or near the fusion site or linker or in the blocking moiety. For example, when the cytokine polypeptide is fused to a blocking moiety through a linker that contains a protease cleavage site, the cytokine polypeptide is released from the blocking moiety and can bind its receptor, upon protease mediated cleavage of the linker. The linker is designed to be cleaved at the site of desired cytokine activity, for example in the tumor microenvironment, avoiding off-target cytokine activity and reducing overall toxicity of cytokine therapy.

[16] The blocking moiety can also function as a serum half-life extension element. In some ~~embodiments~~examples, the fusion polypeptide further comprises a separate serum half-life extension element. In some ~~embodiments~~examples, the fusion polypeptide further comprises a targeting domain. In various ~~embodiments~~examples, the serum half-life extension element is a water-soluble polypeptide such as optionally branched or multi-armed polyethylene glycol (PEG), full length human serum albumin (HSA) or a fragment that preserves binding to FcRn, an Fc fragment, or a nanobody that binds to FcRn directly or to human serum albumin.

[17] In addition to serum half-life extension elements, the pharmaceutical compositions described herein preferably comprise at least one, or more targeting domains that bind to one or more target antigens or one or more regions on a single target antigen. It is contemplated herein that a polypeptide construct of the ~~invention~~disclosure is cleaved, for example, in a disease-specific microenvironment or in the blood of a subject at the protease cleavage site and that the targeting domain(s) will bind to a target antigen on a target cell. At least one target antigen is involved in and/or associated with a disease, disorder or condition. Exemplary target antigens include those associated with a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease.

[18] In some ~~embodiments~~examples, a target antigen is a cell surface molecule such as a protein, lipid or polysaccharide. In some ~~embodiments~~examples, a target antigen is on a tumor cell, virally infected cell, bacterially infected cell, damaged red blood cell, arterial plaque cell, or fibrotic tissue cell.

[19] Target antigens, in some cases, are expressed on the surface of a diseased cell or tissue, for example a tumor or a cancer cell. Target antigens for tumors include but are not limited to Fibroblast activation protein alpha (FAPa), Trophoblast glycoprotein (5T4), Tumor-associated calcium signal transducer 2 (Trop2), Fibronectin EDB (EDB-FN), fibronectin EIIIB domain, CGS-2, EpCAM, EGFR, HER-2, HER-3, c-Met, FOLR1, FAP, and CEA. Pharmaceutical compositions disclosed herein, also include proteins comprising two antigen binding domains that bind to two different target antigens known to be expressed on a diseased cell or tissue. Exemplary pairs of antigen binding domains include but are not limited to EGFR/CEA, EpCAM/CEA, and HER-2/HER-3.

[20] In some ~~embodiments~~examples, the targeting polypeptides independently comprise a scFv, a VH domain, a VL domain, a non-Ig domain, or a ligand that specifically binds to the target antigen. In some ~~embodiments~~examples, the targeting polypeptides specifically bind to a cell surface molecule. In some ~~embodiments~~examples, the targeting polypeptides specifically bind to a tumor antigen. In some ~~embodiments~~examples, the targeting polypeptides specifically and independently bind to a tumor antigen selected from at least one of EpCAM, EGFR, HER-2, HER-3, cMet, CEA, and FOLR1. In some ~~embodiments~~examples, the targeting polypeptides specifically and independently bind to two different antigens, wherein at least one of the antigens is a tumor antigen selected from EpCAM, EGFR, HER-2, HER-3, cMet, CEA, and FOLR1. In some ~~embodiments~~examples, the targeting polypeptide serves as a retention domain and is attached to the cytokine via a non-cleavable linker.

[21] As described herein, the cytokine blocking moiety can bind to the cytokine and thereby block activation of the cognate receptor of the cytokine.

[22] This disclosure also related to nucleic acids, e.g., DNA, RNA, mRNA, that encode the conditionally active proteins described herein, as well as vectors and host cells that contain such nucleic acids.

[23] This disclosure also relates to pharmaceutical compositions that contain a conditionally active protein, nucleic acid that encodes the conditionally active protein, and vectors and host cells that contain such nucleic acids. Typically, the pharmaceutical composition contains one or more physiologically acceptable carriers and/or excipients. In one aspect, the disclosure relates to a pharmaceutical composition comprising: (i) a fusion polypeptide comprising a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H] and a protease-cleavable polypeptide linker; and (ii) a second therapeutic agent; wherein the cytokine polypeptide and the blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker. In another aspect, the disclosure relates to a pharmaceutical composition comprising a (i) a first fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578, and (ii) a second fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578; wherein the first fusion polypeptide and the second fusion polypeptide are not the same. In another aspect, the disclosure relates to a pharmaceutical composition comprising a (i) a first fusion polypeptide comprising a first polypeptide chain comprising an amino acid sequence selected from SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 and a second polypeptide sequence comprising the amino acid sequence of SEQ ID NOs: 263, 264, or 333, and (ii) a second therapeutic agent, wherein the second therapeutic agent is a second fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578; wherein the first fusion polypeptide and the second fusion polypeptide are not the same. In another aspect, the disclosure relates to a method of making a pharmaceutical composition, comprising culturing the host cell of the disclosure in suitable conditions for expression and collection of the fusion polypeptide.

[24] The disclosure also relates to therapeutic methods that include administering to a subject in need thereof an effective amount of a conditionally active protein, nucleic acid that encodes the conditionally active protein, vector or host cells that contain such a nucleic acid, and pharmaceutical compositions of any of the foregoing. Typically, the subject has, or is at risk of developing, a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a

graft-versus-host disease or a host-versus-graft disease.

[25] The disclosure also relates to the use of a conditionally active protein, nucleic acid that encodes the conditionally active protein, vector or host cells that contain such a nucleic acid, and pharmaceutical compositions of any of the foregoing, for treating a subject in need thereof. Typically, the subject has, or is at risk of developing, a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease.

[26] The disclosure also relates to the use of a conditionally active protein, nucleic acid that encodes the conditionally active protein, vector or host cells that contain such a nucleic acid for the manufacture of a medicament for treating a disease, such as a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease. In one aspect, the disclosure relates to a use of the pharmaceutical composition or one or more fusion polypeptides of the disclosure in the manufacture of a medicament for treating a cancer or a viral infection associated with cancer in a subject in need thereof. In another aspect, the disclosure relates to a use of (i) a therapeutically effective amount of a fusion polypeptide comprising a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H] and a protease-cleavable polypeptide linker in the manufacture of a first medicament; and use of (ii) a therapeutically effective amount of a second therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating cancer in a subject in need thereof; wherein the cytokine polypeptide and the cytokine blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker. In another aspect, the disclosure relates to a use of (i) a therapeutically effective amount of a fusion polypeptide comprising a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H] and a protease-cleavable polypeptide linker in the manufacture of a medicament for use in combinatorial therapy for treating a cancer in a subject in need thereof, wherein said medicament is to be administered in combination with (ii) a therapeutically effective amount of a second therapeutic agent; wherein the cytokine polypeptide and the cytokine blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine

receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker. In another aspect, the disclosure relates to a nucleic acid encoding the fusion polypeptide of the disclosure. In another aspect, the disclosure relates to a vector comprising the nucleic acid of the disclosure. In another aspect, the disclosure relates to a host cell comprising the vector of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[27] **FIGs. 1A-1E** are a series of graphs showing activity of IL-2 fusion proteins in a HEKBlue IL-2 reporter assay in the presence of HSA. Squares depict activity of the uncut IL-2 polypeptide (intact) and triangles depict the activity of the cut polypeptide (cleaved). Circles depict activity of control IL-2. EC50 for each IL-2. EC50 values for each are shown in the table. FIGs. 1A-1E also depict results of the protein cleavage assay for each IL-2 polypeptide. Constructs tested and depicted include WW0475 (FIG. 1A), WW0517 (FIG. 1B), WW0548/556 (FIG. 1C), WW0735/523 (FIG. 1D), and WW0621/523 (FIG. 1E). **FIG. 1F** is a graph showing activity of a non-cleavable control, WW0729/523. FIG. 1F also depicts results of a protein cleavage assay for the non-cleavable control.

[28] **FIGs. 2A-2L** are a series of graphs showing activity of fusion proteins in an IL-2 luciferase reporter assay. Closed squares depict activity of the uncut IL-2 polypeptide (intact) and open squares depict the activity of the cut IL-2 polypeptide (cleaved). Circles depict activity of the control (human

CLAIMS

1. A pharmaceutical composition comprising:
 - (i) a fusion polypeptide comprising a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H] and a protease-cleavable polypeptide linker; and
 - (ii) a second therapeutic agent;

wherein the cytokine polypeptide and the blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.
2. The pharmaceutical composition of claim 1, wherein the cytokine polypeptide is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, TGF , interferon alpha, interferon beta, interferon gamma, TNF, TGFbeta, CXCL10, CCL19, CCL20, CCL21, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing.
3. The pharmaceutical composition of claim 2, wherein the cytokine polypeptide is IL-2, IL-12, interferon alpha, interferon beta, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing.
4. The pharmaceutical composition of any one of claims 1-3 wherein the fusion polypeptide has the formula:

[A]-[L1]-[H]-[L2]-[D]
 [D]-[L2]-[H]-[L1]-[A]
 [A]-[L1]-[D]-[L2]-[H]
 [H]-[L2]-[D]-[L1]-[A]
 [H]-[L1]-[A]-[L2']-[D]
 [D]-[L1]-[A]-[L2']-[H]

wherein [A] is a cytokine polypeptide, [D] is a blocking moiety, [H] is a half-life extension moiety, [L1] is a protease-cleavable polypeptide linker, [L2] is an polypeptide linker that is optionally protease-cleavable, and [L2'] is a protease-cleavable polypeptide linker.

5. The pharmaceutical composition of any one of the preceding claims, wherein the second therapeutic agent is a second fusion polypeptide comprising at least one of each of: a second cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension element [H] and a protease-cleavable polypeptide linker [L];

wherein the cytokine polypeptide and the cytokine blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.

6. The pharmaceutical composition of claim 4, wherein the second cytokine polypeptide is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, TGF, interferon alpha, interferon beta, interferon gamma, TNF, TGFbeta, CXCL10, CCL19, CCL20, CCL21, a mutein thereof and active fragments thereof.

7. The pharmaceutical composition of claim 5, wherein the second cytokine polypeptide is IL-2, IL-12, interferon alpha, interferon beta, or a mutein, or an active fragment of any of the foregoing.

8. The pharmaceutical composition of any one of claims 4-6 wherein the second fusion polypeptide has the formula:

[A]-[L1]-[H]-[L2]-[D]

[D]-[L2]-[H]-[L1]-[A]

[A]-[L1]-[D]-[L2]-[H]

[H]-[L2]-[D]-[L1]-[A]

[H]-[L1]-[A]-[L2']-[D]

[D]-[L1]-[A]-[L2']-[H]

wherein [A] is a cytokine polypeptide, [D] is a blocking moiety, [H] is a half-life extension moiety, [L1] is a protease-cleavable polypeptide linker, [L2] is an polypeptide linker that is optionally protease-cleavable, and [L2'] is a protease-cleavable polypeptide linker.

9. The pharmaceutical composition of any one of the preceding claims, wherein the first fusion polypeptide comprises a IL-2 polypeptide and the second fusion polypeptide

comprises a different IL-2 polypeptide, a IL-12 polypeptide, an interferon alpha polypeptide, an interferon beta polypeptide, or a mutein, or an active fragment of any of the foregoing.

10. The pharmaceutical composition of any one of the preceding claims, wherein the first fusion polypeptide comprises a IL-12 polypeptide and the second fusion polypeptide comprises a different IL-12 polypeptide, a IL-2 polypeptide, an interferon alpha polypeptide, an interferon beta polypeptide, or a mutein, or an active fragment of any of the foregoing.

11. The pharmaceutical composition of any one of the preceding claims, wherein the first fusion polypeptide comprises a interferon alpha polypeptide or an interferon beta polypeptide and the second fusion polypeptide comprises a different interferon alpha polypeptide, a different interferon beta polypeptide, a IL-2 polypeptide, a IL-12 polypeptide, or a mutein, or an active fragment of any of the foregoing.

12. The pharmaceutical composition of any one of the preceding claims, further comprising an additional third therapeutic agent.

13. The pharmaceutical composition of any one of the preceding claims, wherein the second therapeutic agent is an agent for treating cancer.

14. The pharmaceutical composition of any of the preceding claims, wherein the second therapeutic agent is an immunomodulator.

15. The pharmaceutical composition of any one of claims 1-14, wherein the second therapeutic agent is a chemotherapeutic agent, radiation therapy, an immunotherapeutic agent, a T cell agonist cytokine, a CAR-T, antibody-drug conjugate, or an oncolytic virus therapy.

16. The pharmaceutical composition of any one of the preceding claims, wherein each protease-cleavable polypeptide linker independently comprises a sequence that is capable of being cleaved by a protease selected from the group consisting of a kallikrein, thrombin, chymase, carboxypeptidase A, cathepsin G, cathepsin L, an elastase, PR-3, granzyme M, a calpain, a matrix metalloproteinase (MMP), an ADAM, a FAP, a cathepsin L, a plasminogen activator, a cathepsin, a caspase, a tryptase, and a tumor cell surface protease.

17. The pharmaceutical composition of any one of the preceding claims, wherein the half-life extension element comprises a serum albumin binding domain, a serum albumin, transferrin, or immunoglobulin Fc, or fragment thereof.
18. The pharmaceutical composition of any one of the preceding claims, wherein the blocking element comprises an antibody or antigen-binding fragment of an antibody that binds to the cytokine polypeptide.
19. The pharmaceutical composition of anyone of the preceding claims, wherein the fusion polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578.
20. The pharmaceutical composition of any one of the preceding claims, wherein the second therapeutic agent is a second fusion protein that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578, and wherein the second fusion protein is not the same.
21. The pharmaceutical composition of any one of the preceding claims, wherein the fusion polypeptide is bonded covalently or noncovalently to a second polypeptide.
22. The pharmaceutical composition of claim 21, wherein the second polypeptide chain and the blocking moiety of the fusion polypeptide are complementary and together form a functional binding site.
23. The pharmaceutical composition of claim 22, wherein the functional binding site has specificity for the cytokine polypeptide contain within the fusional polypeptide.
24. The pharmaceutical composition of any one of claims 21-23, wherein the functional binding site is an Fab fragment of an antibody.
25. The pharmaceutical composition of any one of claims 21-23, wherein the first fusion polypeptide dimerizes.

26. A pharmaceutical composition comprising a (i) a first fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578, and (ii) a second fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578; wherein the first fusion polypeptide and the second fusion polypeptide are not the same.
27. A pharmaceutical composition comprising a (i) a first fusion polypeptide comprising a first polypeptide chain comprising an amino acid sequence selected from SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 and a second polypeptide sequence comprising the amino acid sequence of SEQ ID NOs: 263, 264, or 333, and (ii) a second therapeutic agent, wherein the second therapeutic agent is a second fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578; wherein the first fusion polypeptide and the second fusion polypeptide are not the same.
28. A fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, and 636-646 or an amino acid sequence that has at least about 80% identity to SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, and 636-646.
29. The fusion polypeptide of claim 16, wherein the fusion polypeptide comprises an amino acid sequence selected from SEQ ID NOs. 272, 286, 362, 336, 348, 363, or 580.
30. A polypeptide comprising a first polypeptide that is covalently or non-covalently bonded to a second polypeptide, wherein the first polypeptide comprises an amino acid sequence selected from SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 or an amino acid sequence that has at least 80% identity to the SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 and the second polypeptide sequence comprises an amino acid sequence of SEQ ID NOs: 263, 264, or 333 or an amino acid sequence that has at least 80% identity to SEQ ID NOs: 263, 264, or 333.
31. A fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 368-371, 434-440, 453-519, and 523-538, or an amino acid sequence that has at least about 80% identity to SEQ ID NOs. 368-371, 434-440, 453-519, and 523-538.

32. A fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 421-430, and 539-578, or an amino acid sequence that has at least 80% identity to SEQ ID NOs. 421-430, and 539-578.

33. The fusion polypeptide of claim 33, wherein the fusion polypeptide comprises an amino acid sequence selected from SEQ ID NOs.: 424, 428, 541, 556, 560, 568, or 573.

34. Use of ~~A method for treating a cancer or a viral infection associated with cancer comprising administering to a subject in need thereof~~ the pharmaceutical composition or one or more fusion polypeptides of any one of the preceding claims ~~in the manufacture of a medicament for treating a cancer or a viral infection associated with cancer in a subject in need thereof.~~

35. A nucleic acid encoding the fusion polypeptide of any one of claims 1-27 or 28-33.

36. A vector comprising the nucleic acid of claim 35.

37. A host cell comprising the vector of claim 36.

38. A method of making a pharmaceutical composition, comprising culturing the host cell of claim 37 in suitable conditions for expression and collection of the fusion polypeptide.

39. Use of ~~A combination comprising:~~ (i) a therapeutically effective amount of a fusion polypeptide comprising a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H] and a protease-cleavable polypeptide linker in the manufacture of a first medicament; and use of (ii) a therapeutically effective amount of a second therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating ~~for use in treating a~~ cancer in a subject in need thereof;

wherein the cytokine polypeptide and the cytokine blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.

40. ~~A method of treating a cancer in a subject comprising administering to the subject in need thereof~~ Use of (i) a therapeutically effective amount of a fusion polypeptide comprising a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H] and a protease-cleavable polypeptide linker in the manufacture of a medicament for use in combinatorial therapy for treating a cancer in a subject in need thereof, wherein said medicament is to be administered in combination with; and (ii) a therapeutically effective amount of a second therapeutic agent;

wherein the cytokine polypeptide and the cytokine blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.

41. The use of claim 39 or ~~the method of claim~~ 40, wherein the cytokine polypeptide comprises an amino acid sequence selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, TGF, interferon alpha, interferon beta, Interferon gamma, TNF, TGFbeta, CXCL10, CCL19, CCL20, CCL21, or a mutein, or an active fragment of any of the foregoing.

42. The use ~~or method~~ of claim 41, wherein the cytokine polypeptide is IL-2, IL-12, interferon alpha, interferon beta, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing.

43. The use ~~or method~~ of any one of claims 39-42, wherein the fusion polypeptide has the formula:

[A]-[L1]-[H]-[L2]-[D]

[D]-[L2]-[H]-[L1]-[A]

[A]-[L1]-[D]-[L2]-[H]

[H]-[L2]-[D]-[L1]-[A]

[H]-[L1]-[A]-[L2']-[D]

[D]-[L1]-[A]-[L2']-[H]

wherein A is a cytokine polypeptide, D is a blocking moiety, H is a half-life extension moiety, L1 is a protease-cleavable polypeptide linker, L2 is an polypeptide linker that is optionally protease-cleavable, and L2' is a protease-cleavable polypeptide linker.

44. The use ~~or method~~ of any one of claims 39-43, wherein the second therapeutic agent is a second fusion polypeptide comprising a cytokine polypeptide [A], a blocking moiety [D], optionally a half-life extension moiety [H] and a protease-cleavable polypeptide linker ; wherein the cytokine polypeptide and the cytokine blocking moiety and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the fusion polypeptide is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.

45. The use ~~or method~~ of claim 44, wherein the second cytokine polypeptide is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, TGF , IFN alpha, IFN beta, IFN gamma, TNF, TGFbeta, CXCL10, CCL19, CCL20, CCL21, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing.

46. The use ~~or method~~ of claim 45, wherein the cytokine polypeptide is IL-2, IL-12, interferon alpha, interferon beta or a mutein, or an active fragment of any of the foregoing.

47. The use ~~or method~~ of any one of claims 39-46, wherein the second fusion polypeptide has the formula:

[A]-[L1]-[H]-[L2]-[D]

[D]-[L2]-[H]-[L1]-[A]

[A]-[L1]-[D]-[L2]-[H]

[H]-[L2]-[D]-[L1]-[A]

[H]-[L1]-[A]-[L2']-[D]

[D]-[L1]-[A]-[L2']-[H]

wherein A is a cytokine polypeptide, D is a blocking moiety, H is a half-life extension moiety, L1 is a protease-cleavable polypeptide linker, L2 is an polypeptide linker that is optionally protease-cleavable, and L2' is a protease-cleavable polypeptide linker.

48. The use ~~or method~~ of any one of claims 39-47, wherein the first fusion polypeptide comprises a IL-2 polypeptide and the second fusion polypeptide comprises a different IL-2 polypeptide, a IL-12 polypeptide, an interferon alpha polypeptide, an interferon beta polypeptide, or a mutein, or an active fragment of any of the foregoing.

49. The use ~~or method~~ of any one of claims 39-47, wherein the first fusion polypeptide comprises a IL-12 polypeptide and the second fusion polypeptide comprises a different IL-12 polypeptide, a IL-2 polypeptide, an IFNalpha polypeptide, an interferon beta polypeptide, or a mutein, or an active fragment of any of the foregoing.
50. The use ~~or method~~ of any one of claims 39-50, wherein the first fusion polypeptide comprises a interferon alpha polypeptide or an IFN beta polypeptide and the second fusion polypeptide comprises a different interferon alpha polypeptide, a different interferon beta polypeptide, a IL-2 polypeptide, a IL-12 polypeptide, or a mutein, or an active fragment of any of the foregoing.
51. The use ~~or method~~ of any one of the preceding claims, further comprising an additional third therapeutic agent.
52. The use ~~or method~~ of any one of claims 39-51, wherein the second therapeutic agent is an agent for treating cancer.
53. The use ~~or method~~ of any one of claims 39-52, wherein the second therapeutic agent is an immunomodulator.
54. The use ~~or method~~ of claim any one of claims 39-53, wherein the second therapeutic agent is a chemotherapeutic agent, radiation therapy, an immunotherapeutic agent, a T cell agonist cytokine, a CAR-T, antibody-drug conjugate, or an oncolytic virus therapy.
55. The use ~~or method~~ of any one of claims 39-54, wherein each protease-cleavable polypeptide linker independently comprises a sequence that is capable of being cleaved by a protease selected from the group consisting of a kallikrein, thrombin, chymase, carboxypeptidase A, cathepsin G, cathepsin L, an elastase, PR-3, granzyme M, a calpain, a matrix metalloproteinase (MMP), an ADAM, a FAP, a cathepsin L, a plasminogen activator, a cathepsin, a caspase, a tryptase, and a tumor cell surface protease.

56. The use ~~or method~~ of any one of claims 39-55, wherein the half-life extension element comprises a serum albumin binding domain, a serum albumin, transferrin, or immunoglobulin Fc, or fragment thereof.
57. The use ~~or method~~ of any one of claims 39-56, wherein the cytokine blocking moiety comprises an antibody or an antigen-binding fragment of an antibody that binds the cytokine polypeptide.
58. The use ~~or method~~ of any one of claims 39-56, wherein the first fusion polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578.
59. The use ~~or method~~ of any one of claims 39-57, wherein the second therapeutic agent comprises the amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578.
60. The use ~~or method~~ of any one of claims 39-57, wherein the first fusion polypeptide comprises the amino acid selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578 and the second therapeutic agent comprises the amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 636-646, 579-608, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578, wherein the fusion polypeptide and the second therapeutic agent are different.
61. The use ~~or method~~ of any one of claims 39-60, wherein the first fusion polypeptide is bonded covalently or noncovalently to a second polypeptide chain.
62. The use ~~or method~~ of claim 61, wherein the second polypeptide chain and the blocking moiety of the fusion polypeptide are complementary and together form a functional binding site.
63. The use ~~or method~~ of claim 62, wherein the functional binding site has specificity for the cytokine polypeptide contain within the fusional polypeptide.

64. The use ~~or method~~ of any one of claims 61-63, wherein the functional binding site is an Fab fragment of an antibody.
65. The use ~~or method~~ of any one of claims 61-64, wherein the first fusion polypeptide dimerizes.
66. The ~~method or~~ use of any one of claims 39-65, wherein the first fusion polypeptide comprises a first polypeptide and second polypeptide, wherein the first polypeptide comprises an amino acid sequence selected from SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 and the second polypeptide sequence comprises an amino acid sequence of SEQ ID NOs: 263, 264, or 333
67. The ~~method or~~ use of any one of claims 39-65, comprising a (i) a first fusion polypeptide comprising a first polypeptide chain comprising an amino acid sequence selected from SEQ ID NOs. 362, 363, 325, 286, 579, 581, or 582 and a second polypeptide sequence comprising the amino acid sequence of SEQ ID NOs: 263, 264, or 333, and (ii) a second therapeutic agent, wherein the second therapeutic agent is a second fusion polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 257-300, 302-317, 325-353, 355-365, 366, 372-381, 383-385, 388-420, 579-608, 636-646, 368-371, 434-440, 453-519, 523-538, 421-430, and 539-578; wherein the first fusion polypeptide and the second fusion polypeptide are not the same.
68. The use ~~or method~~ of any one of claims 39-67, wherein the cancer is melanoma, renal cancer, breast cancer, ovarian cancer, prostate cancer, kidney cancer, pancreatic cancer, brain cancer, bladder cancer, or lung cancer.