

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.

[7a] In one aspect, provided herein is a conditionally active IL-2 comprising a fusion polypeptide that is covalently or noncovalently bonded to a second polypeptide, wherein the fusion polypeptide comprises a IL-2 polypeptide [A], a IL-2 blocking moiety [D], a half-life extension moiety [H] and a protease-cleavable polypeptide linker; wherein the second polypeptide and the IL-2 blocking moiety of the fusion polypeptide are complementary and together form a functional binding site for the IL-2 polypeptide that is a Fab fragment of an antibody; wherein the IL-2 blocking moiety is VH-CH1 and the second polypeptide comprises a complementary VL-CL, or the blocking moiety is VL-CL and the second polypeptide comprises a complementary VH-CH1; wherein the half-life extension moiety is human serum albumin or an antigen-binding polypeptide that binds human serum albumin; and wherein the IL-2 polypeptide, the IL-2 blocking moiety and half-life extension moiety are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated IL-2 receptor activating activity, wherein the IL-2 -receptor activating activity of the fusion polypeptide is at least 10-fold less than the IL-2 receptor activating activity of the polypeptide that comprises the IL-2 polypeptide that is produced by cleavage of the protease-cleavable polypeptide linker.

[7b] In another aspect, provided herein is a conditionally active IL-2 comprising a first polypeptide that is covalently or non-covalently bonded to a second polypeptide, wherein: (i) the first polypeptide comprises an amino acid sequence of SEQ ID NO: 286, and the second polypeptide comprises an amino acid sequence of SEQ ID NO: 263; (ii) the first polypeptide comprises the amino acid of SEQ ID NO: 286, and the second polypeptide comprises an amino acid sequence of SEQ ID NO: 264; (iii) the first polypeptide comprises an amino acid sequence of SEQ ID NO: 362, and the second

polypeptide comprises an amino acid sequence of SEQ ID NO: 263; or (iv) the first polypeptide comprises an amino acid sequence of SEQ ID NO: 582, and the second polypeptide comprises an amino acid sequence of SEQ ID NO: 263.

[8] In some examples, the fusion proteins described herein are conditionally active variants of IL-12, IL-2, or IFN. In examples, 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. .

[9] In examples, 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.

[10] In 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.

[11] In some examples, 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 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 complementary 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 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 complementary 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).

[13] In 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

[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 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 conditionally active IL-2.

[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 another aspect, the disclosure relates to a nucleic acid encoding the conditionally active IL-2 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 conditionally active IL-2 comprising a fusion polypeptide that is covalently or noncovalently bonded to a second polypeptide, wherein the fusion polypeptide comprises a IL-2 polypeptide [A], a IL-2 blocking moiety [D], a half-life extension moiety [H] and a protease-cleavable polypeptide linker;

wherein the second polypeptide and the IL-2 blocking moiety of the fusion polypeptide are complementary and together form a functional binding site for the IL-2 polypeptide that is a Fab fragment of an antibody;

wherein the IL-2 blocking moiety is VH-CH1 and the second polypeptide comprises a complementary VL-CL, or the blocking moiety is VL-CL and the second polypeptide comprises a complementary VH-CH1;

wherein the half-life extension moiety is human serum albumin or an antigen-binding polypeptide that binds human serum albumin; and

wherein the IL-2 polypeptide, the IL-2 blocking moiety and half-life extension moiety are operably linked by the protease-cleavable polypeptide linker and the fusion polypeptide has attenuated IL-2 receptor activating activity, wherein the IL-2 -receptor activating activity of the fusion polypeptide is at least 10-fold less than the IL-2 receptor activating activity of the polypeptide that comprises the IL-2 polypeptide that is produced by cleavage of the protease-cleavable polypeptide linker.

2. The conditionally active IL-2 of claim 1 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], or
 [D]-[L1]-[A]-[L2']-[H];

wherein [A] is the IL-2 polypeptide, [D] is the blocking moiety, [H] is the half-life extension moiety, [L1] is a protease-cleavable polypeptide linker, [L2] is a polypeptide linker that is optionally protease-cleavable, and [L2'] is a protease-cleavable polypeptide linker.

3. The conditionally active IL-2 of claim 2, wherein the fusion polypeptide has the formula of [A]-[L1]-[H]-[L2]-[D].

4. The conditionally active IL-2 of any one of the preceding claims, wherein the fusion polypeptide comprises a VH-CH1, and the second polypeptide comprises a complementary VL-CL.
5. The conditionally active IL-2 of any one of the preceding claims, wherein the fusion polypeptide comprises a VL-CL, the second polypeptide comprises a complementary VH-CH1.
6. The conditionally active IL-2 of any one of the preceding claims, wherein the protease cleavable linker comprises an amino acid sequence of SEQ ID: 609.
7. The conditionally active IL-2 of any one of the preceding claims, wherein the IL-2 polypeptide that is produced by cleavage of the protease cleavable linker does not comprise the half-life extension moiety or the IL-2 blocking moiety.
8. The conditionally active IL-2 of any one of the preceding claims, wherein the second polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 263, SEQ ID NO: 264, and SEQ ID NO: 333.
9. The conditionally active IL-2 of any one of the preceding claims, wherein
 - (i) the fusion polypeptide comprises an amino acid sequence selected from SEQ ID NOs.: 286, 325, 362, 363, 579, 581 and 582, or an amino acid sequence that has at least 80% identity to the SEQ ID NOs. 286, 325, 362, 363, 579, 581, or 582; and
 - (ii) the second polypeptide sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 263, 264, and 333, or an amino acid sequence that has at least 80% identity to SEQ ID NO: 263, SEQ ID NO: 264, or SEQ ID NO: 333.
10. The conditionally active IL-2 of any one of the preceding claims, wherein the fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 286, and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 263.
11. The conditionally active IL-2 of any one of the preceding claims, wherein the fusion polypeptide comprises the amino acid of SEQ ID NO: 286, and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 264.
12. The conditionally active IL-2 of any one of the preceding claims, wherein the fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 362, and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 263.

13. The conditionally active IL-2 of any one of the preceding claims, wherein the fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 582, and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 263.
14. A conditionally active IL-2 comprising a first polypeptide that is covalently or non-covalently bonded to a second polypeptide, wherein:
 - (i) the first polypeptide comprises an amino acid sequence of SEQ ID NO: 286, and the second polypeptide comprises an amino acid sequence of SEQ ID NO: 263;
 - (ii) the first polypeptide comprises the amino acid of SEQ ID NO: 286, and the second polypeptide comprises an amino acid sequence of SEQ ID NO: 264;
 - (iii) the first polypeptide comprises an amino acid sequence of SEQ ID NO: 362, and the second polypeptide comprises an amino acid sequence of SEQ ID NO: 263; or
 - (iv) the first polypeptide comprises an amino acid sequence of SEQ ID NO: 582, and the second polypeptide comprises an amino acid sequence of SEQ ID NO: 263.
15. A nucleic acid encoding the conditionally active IL-2 of any one of claims 1-14.
16. A vector comprising the nucleic acid of claim 15.
17. A host cell comprising the vector of claim 16.
18. A method of making a pharmaceutical composition, comprising culturing the host cell of claim 17 in suitable conditions for expression and collection of the conditionally active IL-2.