

CLAIMS

1. A fusion polypeptide comprising at least one of each of:

- a) a cytokine polypeptide [A];
- b) a cytokine blocking moiety [D]; and
- c) a protease-cleavable polypeptide linker [L];

wherein the cytokine polypeptide and the cytokine blocking moiety 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 fusion polypeptide 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 β , IFN α , IFN β , IFN γ , TNF, TGFbeta, CXCL10, CCL19, CCL20, CCL21 and active fragments thereof.

3. The fusion polypeptide of claim 1 or 2, wherein the cytokine polypeptide is a short half-life cytokine polypeptide.

4. The fusion polypeptide of any one of claims 1-3, wherein the protease-cleavable linker polypeptide 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.

5. The fusion polypeptide of any one of claims 1-4 wherein the protease-cleavable polypeptide independently comprises two or more cleavage sites for the same protease, or two or more cleavage sites that are cleaved by different proteases or at least one of the protease-cleavable polypeptides comprises a cleavage site for two or more different proteases.

6. The fusion polypeptide of any one of claims 1-5, wherein the cytokine blocking moiety is also a half-life extension element.

7. The fusion polypeptide of any one of claims 1-6, wherein the cytokine blocking moiety sterically blocks agonist activity of the cytokine.

8. The fusion polypeptide of claim 6 or 7, wherein the cytokine blocking moiety is human serum albumin, an antigen binding protein, or an antigen binding polypeptide which binds human serum albumin, or a fragment thereof.

9. The fusion polypeptide of any one of claims 1-8, wherein the cytokine is free to dissociate from the cytokine blocking moiety after the protease-cleavable polypeptide linker is cleaved by a protease.

10. The fusion polypeptide of any one of claims 1-9, wherein the cytokine blocking moiety inhibits the cytokine polypeptide from activating its cognate receptor.

11. The fusion polypeptide of any of claims 1-10, wherein the fusion polypeptide binds to the cognate receptor of the cytokine polypeptide before cleavage of the protease-cleavable linker.

12. The fusion polypeptide of any one of claims 1-11, further comprising at least one half-life extension element.

13. The fusion polypeptide of any one of claims 1-12, wherein the cytokine blocking moiety non-covalently associates with the cytokine polypeptide, independently of the linker peptide bond.
14. The fusion polypeptide of claim 13, wherein the non-covalent association is pH dependent.
15. The fusion polypeptide of claim 12, wherein the half-life extension element sterically inhibits or blocks activation and/or binding of the cytokine polypeptide to its cognate receptor.
16. The fusion polypeptide of claim 12 wherein the half-life extension element is human serum albumin, a human IgG, a humanized IgG, an scFv, a Fab, an sdAb or a fragment thereof.
17. The fusion polypeptide of claim 1, wherein the cytokine blocking moiety comprises a ligand binding domain, a single domain antibody or scFv that binds the cytokine polypeptide, or an antibody or antibody fragment selected from a single domain antibody and a scFv that binds a receptor of the cytokine polypeptide.
18. The fusion polypeptide of any one of claim 1-17, wherein the cytokine receptor activating activity is determined using a standard *in vitro* receptor activation assay and equal amounts on a mole basis of the cytokine polypeptide and fusion protein.
19. The fusion polypeptide of any one of claims 1-18, wherein cytokine loses contact with the cytokine blocking moiety and/or half-life extension element after the protease-cleavable sequence is cleaved by a protease.
20. The fusion polypeptide of any one of claims 1-19, wherein the fusion polypeptide comprising at least one of each of [A], [D], and [L], in any of the following orientations:
 - a) [A]-[L]-[D];
 - b) [D]-[L]-[A];
 - c) [D]-[L]-[A]-[L]-[D]-[L]-[A];
 - d) [A]-[L]-[D]-[L]-[A]-[L]-[D];
 - e) [D]-[L]-[A]-[L]-[D];
 - f) [D]-[L]-[A]-[L]-[A]-[L]-[D];
 - g) [D]-[L]-[A]-[L]-[D]-[L]-[A]-[L]-[D]; and
 - h) [D]-[L]-[A]-[A]-[L]-[D].
21. The fusion polypeptide of any one of claims 1-20, wherein the fusion polypeptide comprises two cytokine peptides.
22. The fusion polypeptide of claim 1 having the Formula:

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

wherein,

A is a cytokine polypeptide;

L1 and L2 are each independently protease-cleavable polypeptide linkers;

D is a cytokine-blocking moiety optionally capable of extending serum half-life; and

wherein L1 is a substrate for a first protease and wherein L2 is a substrate for a second protease.
23. The fusion polypeptide of any one of claims 12, wherein the half-life extension element is one half-life extension element, two half-life extension elements, or three half-life extension elements.

24. The fusion polypeptide of any one of claims 1-23, comprising one protease-cleavable linker, two protease-cleavable linkers, three protease-cleavable linkers, four protease-cleavable linkers, five protease-cleavable linkers, six protease-cleavable linkers, or seven protease-cleavable linkers.

25. The fusion polypeptide of any one of claims 1-24, further comprising a tumor specific antigen binding peptide.

26. The fusion polypeptide of claim 25, where the tumor specific antigen binding peptide is operably linked to the cytokine polypeptide by a non-cleavable linker.

27. The fusion polypeptide of claim 25, where the tumor specific antigen binding peptide is operably linked to the cytokine polypeptide by a cleavable linker.

28. The fusion polypeptide of any one of claims 1-27, wherein the cytokine polypeptide has a serum half-life comparable to the corresponding naturally occurring cytokine.

29. The fusion polypeptide of any one of claims 1-27, wherein the serum half-life of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the fusion protein is comparable to the corresponding naturally occurring cytokine.

30. The fusion polypeptide of any one of claims 1-27, wherein the cytokine polypeptide is a mutein that has a short serum half-life and is rapidly cleared following cleavage of the fusion polypeptide.

31. A fusion polypeptide comprising at least one of each of:

- a) An interferon polypeptide [A];
- b) An interferon blocking moiety [D]; and
- c) a protease-cleavable polypeptide linker [L]; and

wherein the interferon polypeptide and the interferon blocking moiety are operably linked by the protease-cleavable polypeptide linker the fusion polypeptide has attenuated interferon-receptor activating activity, wherein the interferon-receptor activating activity of the fusion polypeptide is at least about 10X less than the interferon-receptor activating activity of the interferon polypeptide that is produced by cleavage of the protease cleavable linker.

32. The fusion polypeptide of claim 31, having the Formula:

[D]-[L1]-[A]-[L2]-[D];

wherein,

A is an interferon alpha (IFN α) polypeptide;

L1 and L2 are each independently are a protease-cleavable polypeptide linker;

D is an IFN α blocking moiety which also extends in vivo half-life; and

wherein the interferon alpha polypeptide and the interferon alpha blocking moiety are operably linked by the protease-cleavable polypeptide linker the fusion polypeptide has attenuated interferon alpha-receptor activating activity, wherein the interferon alpha-receptor activating activity of the fusion polypeptide is at least about 10X less than the interferon-receptor activating activity of the interferon alpha polypeptide that is produced by cleavage of the protease cleavable linker, and wherein when cleavage of both linkers occurs, in vivo half-life of the interferon alpha polypeptide is substantially similar to that of naturally occurring interferon alpha.

33. The fusion polypeptide of claim 31, having the Formula:

[D]-[L1]-[A]-[L2]-[D]-[L3]-[A]-[L4]-[D];

wherein,
A is an interferon gamma (IFN γ) polypeptide;
L1, L2, L3, and L4 are each independently a protease-cleavable polypeptide linkers;
D is an IFN γ blocking moiety capable of extending serum half-life; and

wherein the interferon gamma polypeptide and the interferon gamma blocking moiety are operably linked by the protease-cleavable polypeptide linker the fusion polypeptide has attenuated interferon gamma-receptor activating activity, wherein the interferon gamma-receptor activating activity of the fusion polypeptide is at least about 10X less than the interferon gamma-receptor activating activity of the interferon gamma polypeptide that is produced by cleavage of the protease cleavable linker, and wherein when cleavage of both linkers occurs, in vivo half-life of the interferon gamma polypeptide is substantially similar to that of naturally occurring interferon gamma.

34. The fusion polypeptide of any one of claims 31-33, wherein the protease-cleavable linker polypeptide 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.

35. The fusion polypeptide of claim 34 wherein each protease-cleavable polypeptide independently comprises two or more cleavage sites for the same protease, or two or more cleavage sites that are cleaved by different proteases or at least one of the protease-cleavable polypeptides comprises a cleavage site for two or more different proteases.

36. The fusion polypeptide of claim 31, wherein the interferon is interferon alpha or interferon gamma.

37. The fusion polypeptide of claim 31 having the Formula:

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

wherein,

A is an interferon polypeptide;

L1 and L2 are each independently protease-cleavable polypeptide linkers;

D is an interferon-blocking moiety optionally capable of extending serum half-life; and

wherein L1 is a substrate for a first protease and wherein L2 is a substrate for a second protease.

38. The fusion polypeptide of any one of claims 31-37, wherein the interferon blocking moiety is also a half-life extension element.

39. The fusion polypeptide of any one of claims 31-37-33, wherein the interferon blocking moiety sterically blocks agonist activity of the cytokine.

40. The fusion polypeptide of claim 38 or claim 39, wherein the interferon blocking moiety is human serum albumin, or an antigen binding polypeptide which binds human serum albumin.

41. The fusion polypeptide of any one of claims 31-40, wherein the interferon is free to dissociate from the interferon blocking moiety after the protease-cleavable polypeptide linker is cleaved by a protease.

42. The fusion polypeptide of any of claims 31-41, wherein the interferon blocking moiety inhibits the interferon polypeptide from activating its cognate receptor.

43. The fusion polypeptide of any of claims 31-42, wherein the fusion polypeptide binds to the cognate receptor for the interferon polypeptide before cleavage of the protease cleavable linker.
44. The fusion polypeptide of any one of claims 31-43, further comprising at least one half-life extension element.
45. The fusion polypeptide of any one of claims 31-44, wherein the interferon blocking moiety non-covalently associates with the interferon polypeptide.
46. The fusion polypeptide of any one of claims 31-44, wherein the noncovalent association is pH dependent.
47. The fusion polypeptide of claim 44, wherein the half-life extension element sterically inhibits or blocks activation and/or binding of the interferon polypeptide to its cognate receptor.
48. The fusion polypeptide of claim 44, wherein the half-life extension element is human serum albumin, a human IgG, or a fragment thereof.
49. The fusion polypeptide of claims 44-48, wherein the half-life extension element does not bind the interferon polypeptide.
50. The fusion polypeptide of claim 31, wherein the interferon blocking moiety comprises a ligand binding domain or fragment of a cognate receptor for the interferon, a single domain antibody or scFv that binds the interferon polypeptide, or an antibody or antibody fragment (e.g., single domain antibody, scFv) that binds a receptor of the interferon.
51. The fusion polypeptide of any one of claims 31-50, wherein the cognate interferon receptor activation activity is determined using a standard *in vitro* receptor activation assay and equal amounts on a mole basis of the fusion polypeptide and interferon polypeptide.
52. The fusion polypeptide of any one of claims 31-51, wherein interferon is free to dissociate from with the interferon blocking moiety and/or half-life extension element after the protease-cleavable sequence is cleaved by a protease.
53. The fusion polypeptide of any one of claims 31-52, wherein the fusion polypeptide comprises at least one of each of [A], [B], and [L], in any of the following orientations:
- a) [A]-[L]-[D];
 - b) [D]-[L]-[A];
 - c) [D]-[L]-[A]-[L]-[D]-[L]-[A];
 - d) [A]-[L]-[D]-[L]-[A]-[L]-[D];
 - e) [D]-[L]-[A]-[L]-[D];
 - f) [D]-[L]-[A]-[L]-[A]-[L]-[D];
 - g) [D]-[L]-[A]-[L]-[D]-[L]-[A]-[L]-[D]; and
 - h) [D]-[L]-[A]-[A]-[L]-[D].
54. The fusion polypeptide of any one of claims 31-53, wherein the fusion polypeptide comprises two cytokine peptides.
55. The fusion polypeptide of claim 54, wherein the two cytokine polypeptides are two different cytokine peptides.

56. The fusion polypeptide of claim 44, wherein the at least one half-life extension element is one half-life extension element or two half-life extension elements.
57. The fusion polypeptide of any one of claims 31-56, comprising one protease-cleavable polypeptide linker is one protease-cleavable linker, two protease-cleavable linkers, three protease-cleavable linkers, four protease-cleavable linkers, five protease-cleavable linkers, six protease-cleavable linkers, or seven protease-cleavable linkers.
58. The fusion polypeptide of any one of claims 31-57 further comprising a tumor specific antigen binding peptide.
59. The fusion polypeptide of claim 58, where the tumor specific antigen binding peptide is operably linked to the interferon polypeptide by a non-cleavable linker.
60. The fusion polypeptide of claim 58, where the tumor specific antigen binding peptide is operably linked to the interferon polypeptide by a cleavable linker.
61. A nucleic acid encoding the fusion polypeptide of any one of claims 1-60.
62. A vector comprising the nucleic acid of claim 61.
63. A host cell comprising the vector of claim 62 or nucleic acid of claim 61.
64. A method of making a pharmaceutical composition, comprising culturing the host cell of claim 63 under suitable conditions for expression and collection of desired polypeptides.
65. A pharmaceutical composition comprising i) a fusion polypeptide of any one of claims 1-60, and ii) a pharmaceutically acceptable excipient.
66. Use of an effective amount of a fusion polypeptide of any one of claims 1-60 in the manufacture of a medicament for treating a tumor in a subject in need thereof.
67. A fusion polypeptide of any one of claims 1-60 for use as a medicament.
68. A fusion polypeptide of any one of claims 1-60 for use in treating a tumor in a subject in need thereof.
69. A pharmaceutical composition for treating a tumor in a subject in need thereof comprising as an active ingredient a fusion polypeptide of any one of claims 1-60.