

CLAIMS

1. A fusion polypeptide of the formula:

[A]-[L1]-[B]-[L2]-[D] or [A]-[L1]-[D]-[L2]-[B] or [D]-[L2]-[B]-[L1]-[A] or
[B]-[L2]-[D]-[L1]-[A] or [D]-[L1]-[B]-[L1]-[A] or [B]-[L1]-[D]-[L1]-[A]

wherein,

A is an interleukin 2 (IL-2) polypeptide;

B is a half-life extension element;

L1 and L2 are each independently a polypeptide linker, wherein L1 is a protease-cleavable polypeptide linker and L2 is optionally a protease-cleavable polypeptide linker;

D is an IL-2 blocking moiety; and

wherein the fusion polypeptide has attenuated IL-2 receptor activating activity, wherein the IL-2-receptor activating activity of the fusion polypeptide is at least about 10X less than the IL-2 receptor activating activity of the polypeptide that contains the IL-2 polypeptide that is produced by cleavage of the protease cleavable linker L1.

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

a) an interleukin 2 (IL-2) polypeptide [A];

b) an IL-2 blocking moiety [D]; and

C) a protease-cleavable polypeptide linker [L]; and

wherein the IL-2 polypeptide and the IL-2 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.

3. The fusion polypeptide of claim 1 or claim 2, wherein the activating activity of the IL-2 polypeptide of the cleaved polypeptide is increased at least about 50X compared to the uncleaved fusion polypeptide.

4. The fusion polypeptide of any one of claims 1-3, wherein the activating activity is assessed using a CTLL-2 proliferation assay, a phospho STAT ELISA, or HEK Blue reporter cell assay.

5. The fusion polypeptide of any one of claims 1-4, wherein the uncleaved fusion polypeptide binds IL-2 receptor alpha (IL-2R α) in a manner substantially similar to the naturally occurring IL-2.

6. The fusion polypeptide of any one of claims 1-5, wherein the blocking moiety inhibits activation of IL-2 receptor alpha/beta/gamma (IL-2R $\alpha\beta\gamma$) and IL-2 receptor beta/gamma (IL-2R $\beta\gamma$) by the IL-2 polypeptide in the uncleaved fusion polypeptide.

7. The fusion polypeptide of any one of claims 1-6, 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), a fibroblast activation

protein (FAP), an ADAM metalloproteinase, a plasminogen activator, a cathepsin, a caspase, a trypsin, and a tumor cell surface protease.

8. The fusion polypeptide of any one of claims 1-7, 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.

9. The fusion polypeptide of any one of claims 1-8, wherein the IL-2 blocking moiety noncovalently binds to the IL-2 polypeptide.

10. The fusion polypeptide of claim 9, wherein the noncovalent binding is pH dependent.

11. The fusion polypeptide of any one of claims 1-10, wherein the IL-2 blocking moiety comprises a ligand binding domain or fragment of a cognate receptor for the IL-2, a single domain antibody, Fab or scFv that binds the IL-2 polypeptide, or an antibody or antibody fragment (e.g., Fab, single domain antibody, scFv) that binds a receptor of the IL-2.

12. The fusion polypeptide of any one of claims 1-11, wherein the IL-2 blocking moiety is also a half-life extension element.

13. The fusion polypeptide of any one of claims 1-8 or 12, wherein the IL-2 blocking moiety sterically blocks agonist activity of the IL-2 polypeptide.

14. The fusion polypeptide of claim 12 or 13, wherein the IL-2 blocking moiety is human serum albumin, or an antigen binding polypeptide which binds human serum albumin.

15. The fusion polypeptide of any of claims 1-14, wherein the IL-2 is free to dissociate from the IL-2 blocking moiety after the protease-cleavable polypeptide linker is cleaved by a protease.

16. The fusion polypeptide of any of claims 1-15, wherein the fusion polypeptide binds IL-2R α .

17. The fusion polypeptide of any one of claims 1-16, further comprising at least one half-life extension element.

18. The fusion polypeptide of claim 17, wherein the half-life extension element is human serum albumin, or an antigen binding polypeptide which binds human serum albumin.

19. The fusion polypeptide of claim 17, wherein the half-life extension element is an immunoglobulin Fc.

20. The fusion polypeptide of claim 13, wherein the IL-2 blocking moiety is human serum albumin, a human IgG, a humanized IgG, a sdAb, a Fab, and scFv or a fragment thereof.

21. The fusion polypeptide of any one of claims 1-20, wherein the IL-2 receptor activation is determined using a standard *in vitro* receptor activation assay and equal amounts on a mole basis of the IL-2 polypeptide and the fusion polypeptide.

22. The fusion polypeptide of any one of claims 1-21, wherein IL-2 is free to dissociate from the IL-2 blocking moiety and/or half-life extension element after the protease-cleavable sequence is cleaved by a protease.

23. The fusion polypeptide of any one of claims 17-19, wherein the at least one half-life extension element is one half-life extension element or two half-life extension elements.

24. The fusion polypeptide of any one of claims 1-23, wherein L2 is a protease-cleavable polypeptide linker.

25. The fusion polypeptide of any one of claims 1-24, wherein L1 is a substrate for a first protease and L2 is a substrate for a second protease.
26. The fusion polypeptide of any one of claims 2-25 further comprising a tumor specific antigen binding peptide.
27. The fusion polypeptide of claim 26 where the tumor specific antigen binding peptide is linked to the IL-2 polypeptide by a non-cleavable linker.
28. The fusion polypeptide of claim 26 where the tumor specific antigen binding peptide is linked to the IL-2 polypeptide, the half-life extension element or the IL-2 blocking moiety by a cleavable linker.
29. The fusion polypeptide of any one of claims 1-28, wherein the serum half-life of the IL-2 polypeptide that is produced by cleavage of the protease cleavable linker is comparable to the half-life of naturally occurring IL-2.
30. The fusion polypeptide of any one of claims 1-29, wherein the IL-2 polypeptide comprise a deletion or substitution of the cystine residue that corresponds to Cys125 of SEQ ID NO:.
31. A nucleic acid encoding the polypeptide of any of claims 1-30.
32. A vector comprising the nucleic acid of claim 31.
33. A host cell comprising the vector of claim 32.
34. A method of making a pharmaceutical composition, comprising culturing the host cell of claim 33 under suitable conditions for expression and collection of desired polypeptides.
35. A pharmaceutical composition comprising i) an effective amount of the fusion polypeptide of any one of claims 1-30, and ii) a pharmaceutically acceptable excipient.
36. Use of an effective amount of a fusion polypeptide of any one of claims 1-30 in the manufacture of a medicament for treating a tumor in a subject in need thereof.
37. A fusion polypeptide of any one of claims 1-30 for use as a medicament.
38. A fusion polypeptide of any one of claims 1-30 for use in treating a tumor in a subject in need thereof.
39. 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 1-30.