

CLAIMS

1. A chimeric protein of a general structure of:

N terminus – (a) – (b) – (c) – C terminus,

wherein:

(a) is a first domain comprising an extracellular domain of a Type I transmembrane protein, which is an immune inhibitory signal, the transmembrane protein being TIGIT,

(b) is a linker comprising at least one cysteine residue capable of forming a disulfide bond, and

(c) is a second domain comprising an extracellular domain of Type II transmembrane protein, which has immune stimulatory properties, the transmembrane protein being selected from 4-1BBL, GITRL, TL1A, and LIGHT, wherein:

the linker connects the first domain and the second domain, comprises a hinge-CH₂-CH₃ Fc domain and optionally further comprises one or more joining linkers, such joining linkers being selected from SEQ ID NOs: 49-95.

2. The chimeric protein of claim 1, wherein the second domain is 4-1BBL.

3. The chimeric protein of claim 1, wherein the second domain is GITRL.

4. The chimeric protein of claim 1, wherein the second domain is TL1A.

5. The chimeric protein of claim 1, wherein the second domain is LIGHT.

6. A chimeric protein of a general structure of:

N terminus – (a) – (b) – (c) – C terminus,

wherein:

(a) is a first domain comprising an extracellular domain of a Type I transmembrane protein, which is an immune inhibitory signal, the transmembrane protein being selected from PD-1, CD172a(SIRPα), and TIGIT,

(b) is a linker comprising at least one cysteine residue capable of forming a disulfide bond, and

(c) is a second domain comprising an extracellular domain of Type II transmembrane protein, which has immune stimulatory properties, the transmembrane protein being LIGHT, wherein:

the linker connects the first domain and the second domain, comprises a hinge-CH₂-CH₃ Fc domain and optionally further comprises one or more joining linkers, such joining linkers being selected from SEQ ID NOs: 49-95.

7. The chimeric protein of claim 6, wherein the first domain is PD-1.

8. The chimeric protein of claim 6, wherein the first domain is CD172a(SIRP α).
9. The chimeric protein of claim 6, wherein the first domain is TIGIT.
10. The chimeric protein of any one of claims 1 to 9, wherein the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, or an antibody sequence.
11. The chimeric protein of any one of claims 1 to 10, wherein the hinge-CH2-CH3 Fc domain is derived from IgG4.
12. The chimeric protein of claim 11, wherein the hinge-CH2-CH3 Fc domain is derived from human IgG4.
13. The chimeric protein of claim 11 or claim 12, wherein the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 46 or SEQ ID NO: 47.
14. The chimeric protein of claim 11 or claim 12, wherein the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 48.
15. The chimeric protein of any one of claims 10 to 14, wherein the linker comprises one or more joining linkers, such joining linkers independently selected from SEQ ID NOs: 49-95.
16. The chimeric protein of claim 15, wherein the linker comprises two or more joining linkers each joining linker independently selected from SEQ ID NOs: 49-95; wherein one joining linker is N terminal to the hinge-CH2-CH3 Fc domain and another joining linker is C terminal to the hinge-CH2-CH3 Fc domain.
17. The chimeric protein of any one of claims 1 to 16, wherein the chimeric protein is a recombinant fusion protein.
18. The chimeric protein of any one of claims 1 to 17, wherein the chimeric protein is capable of forming a stable synapse between cells.
19. The chimeric protein of claim 18, wherein the stable synapse between cells provides spatial orientation that favors tumor reduction.
20. The chimeric protein of claim 18 or claim 19, wherein the spatial orientation positions T cells to attack tumor cells and/or sterically prevents a tumor cell from delivering negative signals, including negative signals beyond those masked by the chimeric protein of the invention.

21. The chimeric protein of any one of claims 1 to 20, wherein the chimeric protein provides sustained negative signal masking effect.
22. The chimeric protein of claim 21, wherein the chimeric protein provides T cell proliferation and allows for anti-tumor attack.
23. The chimeric protein of claim 21 or claim 22, wherein the chimeric protein allows sufficient signal transmission to provide release of stimulatory signals.
24. The chimeric protein of claim 23, wherein the stimulatory signal is a cytokine.
25. The chimeric protein of claim 1, wherein the chimeric protein has an amino acid sequence that is at least 95% identical to the amino acid sequence of one of SEQ ID NOs: 14, 17, or 20.
26. The chimeric protein of claim 6, wherein the chimeric protein has an amino acid sequence that is at least 95% identical to the amino acid sequence of one of SEQ ID NOs: 5, 8, or 11.
27. An expression vector, comprising a nucleic acid encoding the chimeric protein of any one of claims 1 to 26.
28. A host cell, comprising the expression vector of claim 27.
29. A pharmaceutical composition, comprising a therapeutically effective amount of the chimeric protein of any one of claims 1 to 26.
30. Use of a chimeric protein for manufacture of a medicament for treating cancer or an inflammatory disease in a subject in need thereof, wherein the chimeric protein comprises:
- (a) a first domain comprising an extracellular domain of a Type I transmembrane protein, which is an immune inhibitory signal, the transmembrane protein being TIGIT,
 - (b) a linker comprising a hinge-CH2-CH3 Fc domain, and
 - (c) a second domain comprising an extracellular domain of a Type II transmembrane protein, which has immune stimulatory properties, the transmembrane protein selected from 4-1BBL, GITRL, TL1A, and LIGHT.
31. Use of a chimeric protein for manufacture of a medicament for treating cancer or an inflammatory disease in a subject in need thereof, wherein the chimeric protein comprises:

- (a) a first domain comprising an extracellular domain of a Type I transmembrane protein, which is an immune inhibitory signal, the transmembrane protein selected from PD-1, CD172a(SIRPα), and TIGIT,
 - (b) a linker comprising a hinge-CH2-CH3 Fc domain, and
 - (c) a second domain comprising an extracellular domain of Type II transmembrane protein, which has immune stimulatory properties, the transmembrane protein being LIGHT.
32. The use of claims 30 or 31, wherein the subject's T cells are activated when bound by the second domain of the chimeric protein and:
- (a) one or more tumor cells are prevented from transmitting an immunosuppressive signal when bound by the first domain of the chimeric protein,
 - (b) a quantifiable cytokine response in the peripheral blood of the subject is achieved, and/or
 - (c) tumor growth is reduced in the subject in need thereof as compared to a subject treated with antibodies directed to the Type I or Type II protein, or their respective ligands or receptors.
33. The use of any one of claims 30 to 32, wherein the chimeric protein is capable of stimulating the signaling of one or more of LIGHT, 4-1BBL, GITRL, and TL1A and activates antigen-presenting cells.
34. The use of any one of claims 30 or 32 to 33, wherein the chimeric protein is capable of reducing the amount or activity of regulatory T cells (Tregs) as compared to untreated subjects or subjects treated with antibodies directed to the Type I or Type II protein, or their respective ligands or receptors.
35. The use of any one of claims 30 or 32 to 34, wherein the chimeric protein is capable of increasing the priming of effector T cells in draining lymph nodes of the subject as compared to untreated subjects or subjects treated with antibodies directed to the Type I or Type II protein, or their respective ligands or receptors.
36. The use of any of claims 30 or 32 to 35, wherein the chimeric protein is capable of causing an overall decrease in immunosuppressive cells and a shift toward a more inflammatory tumor environment as compared to untreated subjects or subjects treated with antibodies directed to the Type I or Type II protein, or their respective ligands or receptors.
37. The chimeric protein of any one of claims 1 to 26 for use in therapy.