

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, and 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.~~

65. 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.

- ~~76~~. The chimeric protein of claim 65, wherein the first domain is PD-1.

- ~~87~~. The chimeric protein of claim ~~65~~, wherein the first domain is CD172a(SIRPα).
- ~~98~~. The chimeric protein of claim ~~65~~, 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.~~
- ~~119~~. The chimeric protein of any one of claims 1 to ~~108~~, wherein the hinge-CH2-CH3 Fc domain is derived from IgG4.
- ~~1210~~. The chimeric protein of claim ~~119~~, wherein the hinge-CH2-CH3 Fc domain is derived from human IgG4.
- ~~1311~~. The chimeric protein of claim ~~11 or claim 120~~, 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.
- ~~1412~~. The chimeric protein of claim ~~11 or claim 120~~, wherein the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 48.
- ~~1513~~. The chimeric protein of any one of claims 10 to ~~1412~~, wherein the linker comprises one or more joining linkers, such joining linkers independently selected from SEQ ID NOs: 49-95.
- ~~1614~~. The chimeric protein of claim ~~1513~~, 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.
- ~~1715~~. The chimeric protein of any one of claims 1 to ~~1614~~, wherein the chimeric protein is a recombinant fusion protein.
- ~~1816~~. The chimeric protein of any one of claims ~~1-5~~ to ~~1715~~, wherein the chimeric protein is capable of forming a stable synapse between cells.
- ~~1917~~. The chimeric protein of claim ~~1816~~, wherein the stable synapse between cells provides spatial orientation that favors tumor reduction.
- ~~2018~~. The chimeric protein of ~~claim 18 or claim 1917~~, 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~~19. The chimeric protein of any one of claims ~~4~~5 to ~~20~~18, wherein the chimeric protein provides sustained negative signal masking effect.

~~22~~20. The chimeric protein of claim ~~21~~19, wherein the chimeric protein provides T cell proliferation and allows for anti-tumor attack.

~~23~~21. The chimeric protein of claim ~~21~~19 or claim ~~22~~20, wherein the chimeric protein allows sufficient signal transmission to provide release of stimulatory signals.

~~24~~22. The chimeric protein of claim ~~23~~21, wherein the stimulatory signal is a cytokine.

~~25~~23. 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~~24. 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.

25. A nucleic acid encoding the chimeric protein of any one of claims 1 to 24.

~~27~~26. An expression vector, comprising a nucleic acid ~~encoding the chimeric protein of any one of claims 1 to 26~~of claim 25.

~~28~~27. A host cell, comprising the expression vector of claim ~~27~~26.

~~29~~28. A pharmaceutical composition, comprising a therapeutically effective amount of the chimeric protein of any one of claims 1 to ~~26~~24.

~~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~~29. 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.

~~3230~~. The use of claims ~~30 or 31~~29, 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.

~~3331~~. The use of ~~any one of claims 30 to 32~~claim 29 or claim 30, 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.

~~3432~~. The use of any one of claims ~~30 or 32~~29 to ~~33~~31, 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.

~~3533~~. The use of any one of claims ~~30 or 32~~29 to ~~34~~32, 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.

~~3634~~. The use of any of claims ~~30-29 or 32 to 35~~to 33, 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.

~~3735~~. The chimeric protein of any one of claims 1 to ~~26~~24 for use in therapy.