

CLAIMS

What is claimed is:

1. A heterologous chimeric protein comprising:
 - (a) a first domain comprising a portion of colony stimulating factor 1 receptor (CSF1R) that is capable of binding a CSF1R ligand;
 - (b) a second domain comprising a portion of CD40 Ligand (CD40L) that is capable of binding a CD40L receptor; and
 - (c) a linker linking the first domain and the second domain.
2. The heterologous chimeric protein of claim 1, wherein the first domain comprises substantially all of the extracellular domain of CSF1R and the second domain comprises substantially all of the extracellular domain of CD40L.
3. The heterologous chimeric protein of claim 1 or claim 2, wherein the chimeric protein is capable of inhibiting an immunosuppressive signal.
4. The heterologous chimeric protein of any one of claims 1 to 3, wherein the chimeric protein is capable of:
 - (a) reducing or eliminating an immune inhibitory signal when the portion of CSF1R is bound to its ligand and/or
 - (b) increasing or activating an immune stimulatory signal when the portion of CD40L is bound to its receptor.
5. The heterologous chimeric protein of any one of claims 1 to 4, wherein the CSF1R ligand is CSF1 or IL-34.
6. The heterologous chimeric protein of any one of claims 1 to 5, wherein the CD40L receptor is CD40.
7. The heterologous chimeric protein of any one of claims 1 to 6, wherein the chimeric protein is capable of contemporaneously binding the CSF1R ligand and the CD40L receptor, wherein the CSF1R ligand is CSF1 or IL-34 and the CD40L receptor is CD40.
8. The heterologous chimeric protein of any one of claims 1 to 7, wherein the chimeric protein is capable of contemporaneously binding recombinant human CD40 and human CSF1 *in vitro*.
9. The heterologous chimeric protein of any one of claims 1 to 8, wherein the chimeric protein depletes CSF1 and/or IL-34, optionally in the serum.

10. The heterologous chimeric protein of any one of claims 1 to 9, wherein the chimeric protein exhibits enhanced safety profiles and/or reduced toxicity profiles compared to CD40 agonist antibodies and/or CSF1R antagonistic antibodies.
11. The heterologous chimeric protein of any one of claims 1 to 10, wherein the chimeric protein exhibits enhanced anti-tumor effects compared to CD40 agonist antibodies and/or CSF1R antagonistic antibodies.
12. The heterologous chimeric protein of any one of claims 1 to 11, wherein the chimeric protein is capable of increasing or preventing a decrease in a sub-population of CD4+ and/or CD8+ T cells.
13. The heterologous chimeric protein of any one of claims 1 to 12, wherein the chimeric protein is capable of enhancing tumor killing activity by T cells.
14. The heterologous chimeric protein of any one of claims 1 to 13, wherein the chimeric protein is capable of providing a sustained immunomodulatory effect.
15. The heterologous chimeric protein of any one of claims 1 to 14, wherein the chimeric protein is capable of causing activation of antigen presenting cells.
16. The heterologous chimeric protein of any one of claims 1 to 15, wherein the chimeric protein is capable enhancing the ability of antigen presenting cells to present antigen.
17. The heterologous chimeric protein of any one of claims 1 to 16, wherein the chimeric protein shifts the ratio of immune cells in favor of cells that can kill a tumor in opposition to cells that protect tumors.
18. The heterologous chimeric protein of claim 17, wherein the cell that that can kill a tumor is selected from T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, anti-tumor macrophages (e.g., M1 macrophages), B cells, and dendritic cells and wherein the cell that protect tumors is selected from myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs); tumor associated neutrophils (TANs), M2 macrophages, and tumor associated macrophages (TAMs)).
19. The heterologous chimeric protein of claim 18, wherein the chimeric protein stimulates anti-tumor macrophages and antigen presenting cells, while avoiding induction of MDSC through inhibition of CSF1 and/or IL-34.
20. The heterologous chimeric protein of any one of claims 1 to 19, wherein the chimeric protein increases the ratio of effector T cells to regulatory T cells.
21. The heterologous chimeric protein of any one of claims 1 to 20, wherein the chimeric protein provides a sustained masking effect of immune inhibitory signals.

22. The heterologous chimeric protein of any one of claims 1 to 21, wherein the chimeric protein provides longer on-target (e.g., intra-tumoral) half-life ($t_{1/2}$) as compared to serum $t_{1/2}$ of the chimeric proteins.
23. The heterologous chimeric protein of any one of claims 1 to 22, wherein the chimeric protein reduces toxicities as compared with treatment with antibodies against CSF1R and CD40.
24. The heterologous chimeric protein of any one of claims 1 to 23, wherein the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, or an antibody sequence.
25. The heterologous chimeric protein of claim 24, wherein the linker comprises hinge-CH2-CH3 Fc domain derived from IgG4.
26. The heterologous chimeric protein of claim 25, wherein the hinge-CH2-CH3 Fc domain is derived from human IgG4.
27. The heterologous chimeric protein of any one of claims 1 to 26, wherein the chimeric protein is expressed by a mammalian host cell as a secretable and functional single polypeptide chain.
28. The heterologous chimeric protein of any one of claims 1 to 27, wherein the portion of CSF1R is at least 95% identical to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.
29. The heterologous chimeric protein of any one of claims 1 to 28, wherein the portion of CD40L is at least 95% identical to the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 4.
30. The heterologous chimeric protein of any one of claims 1 to 29, wherein the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 27.
31. The heterologous chimeric protein of any one of claims 1 to 30, wherein the linker comprises one or more joining linkers, such joining linkers independently selected from SEQ ID NOs: 28 to 74.
32. The heterologous chimeric protein of claim 31, wherein the linker comprises two or more joining linkers each joining linker independently selected from SEQ ID NOs: 28 to 74; 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.
33. The heterologous chimeric protein of any one of claims 1 to 32, wherein the chimeric protein is a recombinant fusion protein.
34. The heterologous chimeric protein of any one of claims 1 to 33, wherein the chimeric protein is capable of forming a stable synapse between cells.

35. The heterologous chimeric protein of claim 34, wherein the stable synapse between cells provides spatial orientation that favors tumor reduction.
36. The heterologous chimeric protein of claim 34 or claim 35, 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.
37. The heterologous chimeric protein of any one of claims 1 to 36, wherein binding of either or both of the extracellular domains to its respective binding partner occurs with slow off rates (K_{off}), which provides a long interaction of a receptor and its ligand.
38. The heterologous chimeric protein of claim 37, wherein the long interaction delivers a longer positive signal effect.
39. The heterologous chimeric protein of claim 38, wherein the longer positive signal effect allows an effector cell to be adequately stimulated for an anti-tumor effect.
40. The heterologous chimeric protein of any one of claims 37 to 39, wherein the long interaction provides T cell proliferation and allows for anti-tumor attack.
41. The heterologous chimeric protein of any one of claims 37 to 40, wherein the long interaction allows sufficient signal transmission to provide release of stimulatory signals.
42. The heterologous chimeric protein of claim 41, wherein the stimulatory signal is a cytokine.
43. An expression vector, comprising a nucleic acid encoding the chimeric protein of any one of claims 1 to 42.
44. A host cell, comprising the expression vector of claim 43.
45. A pharmaceutical composition, comprising a therapeutically effective amount of the heterologous chimeric protein of any one of claims 1 to 42.
46. A method of treating cancer or an inflammatory disease, comprising administering an effective amount of the pharmaceutical composition of claim 45 to a subject in need thereof.
47. A method of modulating a patient's immune response, comprising administering an effective amount of the pharmaceutical composition of claim 45 to a subject in need thereof.
48. The method of claim 46 or claim 47, wherein the patient's T cells are activated.

49. The method of claim 46 or claim 47, wherein the patient has a tumor and one or more tumor cells are prevented from transmitting an immunosuppressive signal.

50. A method for treating cancer or an inflammatory disease comprising administering an effective amount of a pharmaceutical composition to a subject in need thereof, the pharmaceutical composition comprising a heterologous chimeric protein comprising:

- (a) a first domain comprising a portion of colony stimulating factor 1 receptor (CSF1R) that is capable of binding a CSF1R ligand,
- (b) a second domain comprising a portion of CD-40 ligand (CD40L) that is capable of binding an CD40L receptor, and
- (c) a linker linking the first domain and the second domain.

51. The method of claim 50, wherein the subject's T cells are activated when bound by the second domain of the heterologous chimeric protein and:

- (a) one or more tumor cells are prevented from transmitting an immunosuppressive signal when bound by the first domain of the heterologous 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 CD40 blocking antibodies and/or CSF1 or IL-34 blocking antibodies.

52. The method of claim 50 or claim 51, wherein the method inhibits CSF1R signaling and inhibits of suppressive myeloid cell populations.

53. The method of any one of claims 50 to 52, wherein the method stimulates CD40 signaling and activates antigen-presenting cells.

54. The method of any one of claims 50 to 53, wherein the method reduces the amount or activity of tumor associated macrophages (TAMs) as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R.

55. The method of any one of claims 50 to 54, wherein the method reduces the amount or activity of tumor associated macrophages (TAMs) in the tumor microenvironment (TME) as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R.

56. The method of any one of claims 50 to 55, wherein the method reduces the amount or activity of regulatory T cells (Tregs) as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R.

57. The method of any one of claims 50 to 56, wherein the method reduces the amount or activity of IL-10 and/or IL-4 as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R.

58. The method any of one of claims 50 to 57, wherein the method increases maturation and differentiation of proinflammatory macrophages and dendritic cells as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R.

59. The method of any one of claims 50 to 58, wherein the method increases priming of effector T cells in draining lymph nodes of the subject as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R.

60. The method of any one of claims 50 to 59, wherein the method causes an overall decrease in immunosuppressive cells and a shift toward a more inflammatory tumor environment as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R.

61. The chimeric protein of any one of claims 1 to 42 for use as a medicament.

62. The chimeric protein of any one of claims 1 to 42 for use in the treatment of cancer.

63. The chimeric protein of any one of claims 1 to 42 for use in the treatment of an inflammatory disease.

64. Use of the chimeric protein of any one of claims 1 to 42 in the manufacture of a medicament.

65. A recombinant fusion protein comprising a general structure of:

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

wherein:

(a) is a first domain comprising an extracellular domain of CSF1R that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2 and is capable of binding a CSF1R ligand,

(b) is a linker linking the first domain and the second domain and comprising a hinge-CH2-CH3 Fc domain derived from human IgG4, and optionally a joining linker sequence from SEQ ID 28 to 74, and

(c) is a second domain comprising an extracellular domain of CD40 ligand (CD40L) that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4 and is capable of binding an CD40L receptor.

66. The recombinant fusion protein of claim 65, wherein the linker comprises a sequence which is at least 95% identical to the amino acid sequence of SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 27.

67. The recombinant fusion protein of claim 65 or claim 66 for use as a medicament.

68. The recombinant fusion protein of any one of claims 65 to 67 for use in the treatment of cancer.

69. The recombinant fusion protein of any one of claims 65 to 68 for use in the treatment of an inflammatory disease.

70. Use of the recombinant fusion protein of any one of claims 65 to 69 in the manufacture of a medicament.