CLAIMS

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, wherein the linker comprises:

(i) a hinge-CH2-CH3 Fc domain, and

(ii) 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.

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 1-23, wherein the hinge-CH2-CH3 Fc domain is derived from IgG4, optionally wherein the hinge-CH2-CH3 Fc domain is derived from human IgG4.

25. The heterologous chimeric protein of any one of claims 1 to 24, 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.

26. The heterologous chimeric protein of any one of claims 1 to 25, 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.

27. The heterologous chimeric protein of any one of claims 1 to 26, 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.

28. The heterologous chimeric protein of any one of claims 1 to 27, wherein the chimeric protein is a recombinant fusion protein.

29. The heterologous chimeric protein of any one of claims 1 to 28, wherein the chimeric protein is capable of forming a stable synapse between cells.

30. The heterologous chimeric protein of claim 29, wherein the stable synapse between cells provides spatial orientation that favors tumor reduction.

31. The heterologous chimeric protein of claim 29 or claim 30, 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.

32. A nucleic acid encoding the chimeric protein of any one of claims 1 to 31.

33. An expression vector, comprising a nucleic acid of claim 32.

34. A host cell, comprising the expression vector of claim 33.

35. A pharmaceutical composition, comprising a therapeutically effective amount of the heterologous chimeric protein of any one of claims 1 to 31.

36. Use of a heterologous chimeric protein in manufacture of a medicament for treating cancer or an inflammatory disease in a subject in need thereof, wherein the heterologous chimeric protein comprises:

(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, wherein the linker comprises:

(i) a hinge-CH2-CH3 Fc domain, and

(ii) 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.

37. The use of claim 36, 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.

38. The use of claim 36 or claim 37,

wherein the heterologous chimeric protein is capable of inhibiting the CSF1R signaling and inhibits of suppressive myeloid cell populations, and/or

wherein the heterologous chimeric protein is capable of stimulating the CD40 signaling and activates antigen- presenting cells, and/or

wherein the heterologous chimeric protein is capable of reducing the amount or activity of tumor associated macrophages (TAMs) as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R, and/or

wherein the heterologous chimeric protein is capable of reducing 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, and/or

wherein the heterologous chimeric protein is capable of reducing the amount or activity of regulatory T cells (Tregs) as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R, and/or

wherein the heterologous chimeric protein is capable of reducing 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, and/or

wherein the heterologous chimeric protein is capable of increasing the maturation and differentiation of proinflammatory macrophages and dendritic cells as compared to untreated subjects or subjects targeting one of CD40/CD40L and CSF1/CSF1R, and/or

wherein the heterologous 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 targeting one of CD40/CD40L and CSF1/CSF1R, and/or

wherein the heterologous 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 targeting one of CD40/CD40L and CSF1/CSF1R.

39. The chimeric protein of any one of claims 1 to 31 for use in therapy.

40. 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 a second domain, wherein the linker comprises:

(i) a hinge-CH2-CH3 Fc domain derived from human IgG4, and

(ii) a joining linker sequence selected from SEQ ID 28 to 74; and

(c) is the 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.

41. The recombinant fusion protein of claim 40, 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.

42. The recombinant fusion protein of claim 40 or claim 41 for use in therapy.