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SIRP1A - and CD40L-BASED CHIMERIC PROTEINS**TECHNICAL FIELD**

The present technology relates to, *inter alia*, compositions and methods, including chimeric proteins that find use in the treatment of disease, such as immunotherapies for cancer comprising doses, dosing regimens that including biphasic dosing or dosing regimens comprising three cycles.

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PRIORITY

This application claims the benefit of, and priority to, U.S. Provisional Application No. 63/278,567, filed November 12, 2021; and U.S. Provisional Application No. 63/371,083, filed August 11, 2022, the contents of each of which are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

15 The instant application contains a sequence listing, which has been submitted in XML format via EFS-Web. The contents of the XML copy named "SHK-055PC_116981-5055_Sequence_Listing," which was created on November 9, 2022, and is 82,547 bytes in size, and the contents of which are incorporated herein by reference in their entirety.

BACKGROUND

20 The field of cancer immunotherapy has grown tremendously over the past several years. This has been largely driven by the clinical efficacy of antibodies targeting the family of checkpoint molecules (e.g., CTLA-4 and PD-1/L1) in many tumor types. However, despite this success, clinical response to these agents as monotherapy occurs in a minority of patients (10-45% in various solid tumors), and these therapies are hindered by side effects.

25 Rational dose selection and optimization of dosing regimens are of clinical importance and are prerequisites for enhancing patients' medication compliance and obtaining maximum clinical benefits. Developing dosing regimen typically relies on pharmacokinetic/pharmacodynamic studies performed in animal models. However, since immunotherapy does not exert direct anti-proliferative activity on cancer cells, but are instead expected to harness tumor immunity, typically murine surrogates are used for some animal studies, making
30 the pharmacokinetic/pharmacodynamic studies not available. In addition, physicians may vary the dosing regimen of immunotherapy based on the immunogenicity of a tumor, disease stage, and physical status of patients. Therefore, novel strategies to develop dosing and regimens are required.

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SUMMARY

Accordingly, in various aspects, the present disclosure provides compositions and methods that are useful for developing strategies for developing doses and dosing regimen of cancer immunotherapy. The present disclosure is based, in part, on the discoveries that CD80+ cells, CD8+ cells, Granzyme B+ cells, CD68+ cells, Ki67+ cells, and PD-L1+ immune cells increase in the tumor microenvironment (TME) following the administration of the SIRP α -Fc-CD40L chimeric protein; B cells and/or CD40+ cells marginate from peripheral blood within hours of dosing and return to peripheral blood within days; and that the levels of certain serum cytokines increase within hours of dosing and decrease within days following the administration of the SIRP α -Fc-CD40L chimeric protein.

An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level of a cell has been measured in a pre-dose biological sample obtained from the subject before the administration of the first dose, wherein the marker is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell. In embodiments, the method further comprises administering to the human subject a second dose of the chimeric protein if a post-dosing level of the cell is greater than the background level of the cell. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose. In embodiments, the biological sample is a biopsy sample or a surgical specimen. In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the level of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising

5 an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level and/or activity of B cells and/or CD40+ cells has been measured in a first biological sample obtained from the subject before the administration of the first dose. In embodiments, an N-hr post-dose level and/or activity of B cells and/or CD40+ cells has been measured in a second biological sample obtained from the subject after the administration of the first dose. In embodiments, N is a number between 1 and 24. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, an M-day post-dose level and/or activity of B cells and/or CD40+ cells has been measured in a third biological sample obtained from the subject after the administration of the first dose. In embodiments, M is a number between 1 and 28. In embodiments, the method further comprises (ii) administering to the human subject a second dose of the chimeric protein if the M-day post-dose level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood. In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background amount and/or activity of a cytokine selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC has been measured in a first biological sample obtained from the subject before the administration of the first dose. In embodiments, a N-hr post-dose amount and/or activity of the cytokine has been measured in a second biological sample obtained from the subject after the administration of the first dose. In embodiments, N is a number between 1 and 24. In embodiments, the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine. In embodiments, the M-day post-dose amount and/or activity of the cytokine has been measured in a third biological sample obtained from the subject after the administration of the first dose. In embodiments, M is a number between

5 1 and 28. In embodiments, the method further comprises (ii) administering to the human subject a second dose of the chimeric protein if the M-day post-dose amount and/or activity of the cytokine is at least about 30% lower than the N-hr post-dose amount and/or activity of the cytokine. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood. In embodiments, 10 the amount and/or activity of the cytokine is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

An aspect of the present disclosure is a method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer. In embodiments, the method comprises: (i) 15 obtaining a biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 20 ligand (CD40L). In embodiments, a background level and/or activity of a cell has been measured in a biological sample obtained from the subject before the administration of the first dose. In embodiments, the cell is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell. In embodiments, the method further comprises (ii) determining a post-dose level and/or activity of the cell in the biological sample. In embodiments, the method further comprises (iii) 25 determining that the chimeric protein is efficacious if the post-dose level and/or activity of the cell is greater than the background level and/or activity of the cell. In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the level and/or activity of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

30 An aspect of the present disclosure is a method of selecting a subject for treatment with a therapy for a cancer. In embodiments, the method comprises: (i) obtaining a biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second 35 domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising

5 an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level and/or activity of a cell has been measured in a biological sample obtained from the subject before the administration of the first dose. In embodiments, the cell is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell. In embodiments, the method further comprises (ii) determining a post-dose level and/or activity of the cell in the biological sample. In
10 embodiments, the method further comprises (iii) selecting the subject for treatment with the chimeric protein if the post-dose level and/or activity of the cell is greater than the background level and/or activity of the cell. In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the level and/or activity of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell
15 sorting (FACS) or a combination thereof.

An aspect of the present disclosure is a method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer. In embodiments, the method comprises: (i) obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C
20 terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the method further comprises determining a background level and/or activity of B cells and/or CD40+ cells in the first biological sample. In embodiments, the method further
25 comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose level and/or activity of B cells and/or CD40+ cells in the second biological sample. In embodiments, the method further comprises (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises
30 determining an M-day post-dose level and/or activity of B cells and/or CD40+ cells in the third biological sample. In embodiments, the method further comprises (v) determining that the chimeric protein is efficacious if the N hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells, and/or if the M day post-dose level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells
35 and/or CD40+ cells. In embodiments, the first biological sample, the second biological sample and the third

5 biological sample are blood. In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

An aspect of the present disclosure is a method of selecting a subject for treatment with a therapy for a cancer. In embodiments, the method comprises: (i) obtaining a first biological sample obtained from the
10 subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the method further comprises
15 determining a background level and/or activity of B cells and/or CD40+ cells in the first biological sample. In embodiments, the method further comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose level and/or activity of B cells and/or CD40+ cells in the second biological sample. In embodiments, the method further comprises (iv) obtaining a third biological sample
20 obtained from the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises determining an M-day post-dose level and/or activity of B cells and/or CD40+ cells in the third biological sample. In embodiments, the method further comprises (v) selecting the subject for treatment with the chimeric protein if the N hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells, and/or if the M day post-dose
25 level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood. In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a
30 combination thereof.

An aspect of the present disclosure is a method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer. In embodiments, the method comprises: (i) obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C
35 terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein

5 α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the method further comprises (ii) determining in the first biological sample a background amount and/or activity of a cytokine. In embodiments, the cytokine is selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC. In embodiments, the method further comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose amount and/or activity of the cytokine in the second biological sample. In embodiments, the method further comprises (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises determining an M-day post-dose amount and/or activity of the cytokine in the third biological sample. In embodiments, the method further comprises (v) determining that the chimeric protein is efficacious if the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine, and/or if the M-day post-dose amount and/or activity of the cytokine is at least about 50% lower than the N-hour post-dose amount and/or activity of the cytokine. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood. In embodiments, the amount and/or activity of the cytokine is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

An aspect of the present disclosure is a method of selecting a subject for treatment with a therapy for a cancer. In embodiments, the method comprises: (i) obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the method further comprises (ii) determining in the first biological sample a background amount and/or activity of a cytokine. In embodiments, the cytokine is selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC. In embodiments, the method further comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose amount and/or activity of the cytokine in the second biological

5 sample. In embodiments, the method further comprises (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises determining an M-day post-dose amount and/or activity of the cytokine in the third biological sample. In embodiments, the method further comprises (v) selecting the subject for treatment with the chimeric protein if the N-hr post-dose amount and/or activity of the cytokine is greater than the background
10 amount and/or activity of the cytokine, and/or if the M-day post-dose amount and/or activity of the cytokine is at least about 50% lower than the N-hour post-dose amount and/or activity of the cytokine. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood. In embodiments, the amount and/or activity of the cytokine is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and
15 fluorescent activating cell sorting (FACS) or a combination thereof.

In embodiments, the first domain is capable of binding a CD172a (SIRPα) ligand. In embodiments, the first domain comprises substantially all of the extracellular domain of CD172a (SIRPα). In embodiments, the first domain comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence
20 of SEQ ID NO: 57.

In embodiments, the second domain is capable of binding a CD40 receptor. In embodiments, the second domain comprises substantially all of the extracellular domain of CD40L. In embodiments, the second domain comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of
25 SEQ ID NO: 58.

In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of
30 SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, (a) the first domain comprises the amino acid sequence of SEQ ID NO: 57, (b) the second domain comprises the amino acid sequence of SEQ ID NO: 58, and (c) the linker comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID

5 NO: 2, or SEQ ID NO: 3. In embodiments, the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7. In embodiments, the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 and SEQ ID NO: 7.

In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%
10 identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61.

In embodiments, the first dose of the chimeric protein is in the range of from about 0.03 mg/kg to 10 mg/kg. In embodiments, the first dose is about 0.003, or about 0.01, or about 0.03, or about 0.1, or about 0.3, or about 1, or about 2, or about 3, or about 4, or about 6, or about 8, or about 10 mg/kg.

In embodiments, the method of any of the aspects disclosed herein further comprises administration of a
15 second dose of the chimeric protein. In embodiments, the second dose is administered at least about 3 days, or at least about 4 days, or at least about 5 days, or at least about 6 days, or at least about 7 days, or at least about 8 days, or at least about 9 days, or at least about 10 days, or at least about 14 days, or at least about 21 days, or at least about 28 days after the administration of the first dose. In embodiments, the second dose of the chimeric protein is in the range of from about 0.03 mg/kg to 10 mg/kg. In embodiments, the second
20 dose is about 0.003, or about 0.01, or about 0.03, or about 0.1, or about 0.3, or about 1, or about 2, or about 3, or about 4, or about 6, or about 8, or about 10 mg/kg.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

25 **FIG. 1** shows the diagrammatic representation of the SIRPα-Fc-CD40L chimeric protein (SL-172154). SL-172154 (SIRPα-Fc-CD40L) is a hexameric, bi-functional fusion protein consisting of SIRPα (binding affinity to CD47 is 0.628 nM) linked to CD40L (binding affinity to CD40 is 4.74 nM) through an Fc linker protein

FIG. 2 shows the Dose Escalation per modified Toxicity Probability (mTPI-2) Design (N=15). The planned dose escalation is in half-log increments. At least 3 subjects were enrolled into sequential dose levels (DL)
30 and evaluated for dose limiting toxicity (DLT) in the first cycle of treatment. Subjects receive intravenous (IV) administration of SL-172154 on Schedule 1 or Schedule 2 until disease progression, unacceptable toxicity, or withdrawal of consent.

5 **FIG. 3** shows the tumor response and duration of treatment. iUPD = unconfirmed progressive disease (iRECIST) NE = non-evaluable; PD = progressive disease; SD = stable disease

FIG. 4A to **FIG. 4D** show the reproducible increases in serum cytokines following repeated dosing of SL-172154. The changes in the levels of CCL2 (MCP-1) (**FIG. 4A**), CCL4 (MIP-1 β) (**FIG. 4B**), CCL3 (MIP-1 α) (**FIG. 4C**), and CCL22 (MDC) (**FIG. 4D**) are shown.

10 **FIG. 5A** and **FIG. 5B** show the dose-dependent and reproducible increases in Serum IL-12. **FIG. 5A** shows the subject level interleukin 12 (IL-12), a mediator of TH1 proinflammatory responses over time typify the cyclic effector cytokine responses observed in study subjects. **FIG. 5B** shows median responses at the first infusion (horizontal bars), preliminarily appear to be dose dependent.

FIG. 6A and **FIG. 6B** demonstrate the SL-172154 preferentially binds CD47 on leukocytes but not RBCs.

15 **FIG. 6A** shows the CD47 receptor occupancy (RO) as evaluated by fluorescence activated cell sorting (FACS) analysis using whole blood on both red blood cells (RBC) and white blood cells (WBC, leukocytes). At one-hour post infusion on Cycle 1 Day 1 (C1D1), median CD47 RO on leukocytes (horizontal bars) is ~80%. **FIG. 6B** shows the CD47 RO on RBC is <5% for all dose levels.

FIG. 7A to **FIG. 7C** demonstrate that SL-172154 stimulates dose-dependent B cell margination and activation. **FIG. 7A** shows the median frequency of marginating cells increases in a dose-dependent manner (horizontal bars). Receptor engagement is ~100% at all dose levels (data not shown). **FIG. 7B** shows the median B cell frequencies return to pre-infusion levels by the next infusion, maintaining a cyclic pattern of egress and return with each infusion cycle. **FIG. 7C** shows the returning B cells exhibit increases in the co-stimulatory marker CD86, as well as the maturation marker CD95, suggesting that SL-172154 can induce
25 phenotypic changes.

FIG. 8A and **FIG. 8B** show the distinct profile of TNF α and Interleukin-6 (IL-6) relative to CD40 mAbs. **FIG. 8A** shows the induction of TNF α at various doses of CP-870,893 (left panel) or SL-172154 (right panel). **FIG. 8B** shows the induction of IL-6 at various doses of CP-870,893 (left panel) or SL-172154 (right panel). The CP-870,893 data are from Vonderheide *et al.*, *J Clin Oncol* 25:876-883 (2007).

30 **FIG. 9A** and **FIG. 9B** demonstrate that SL-172154 induces innate immune response in tumor microenvironment (TME). **FIG. 9A** shows the immunohistochemistry analysis of biopsy sample from patient A before and after the administration of SL-172154. Monocytes were detected by staining for CD68 (a protein highly expressed by cells in the monocyte lineage). **FIG. 9B** demonstrates the upregulation of activation

5 markers, CD40 and MHC Class II in TME in tumor biopsy sample after the treatment with SL-172154, compared to pretreatment biopsy samples.

FIG. 10A and **FIG. 10B** demonstrate that SL-172154 induces adaptive immune response in tumor microenvironment (TME). **FIG. 10A** shows the CD8+ cells, Granzyme B+ cells, CD68+ cells, and Ki67+ cells in biopsy sample from patient A before and after the administration of SL-172154. The CD8+ cells, Granzyme B+ cells, CD68+ cells, and Ki67+ cells are increased in post-treatment biopsy sample, compared to pre-treatment biopsy sample. **FIG. 10B** is a plot comparing the tumor proportion score (TPS) and combined positive score (CPS).

FIG. 11 shows the planned clinical development strategy for SL-172154. The strategy includes a trial for SL-172154 monotherapy in ovarian cancer, combination therapy of SL-172154 + liposomal doxorubicin in ovarian cancer, combination therapy of SL-172154 + azacitidine + venetoclax in AML, combination therapy of SL-172154 + azacitidine in HR-MDS, and combination therapy of SL-172154 + azacitidine in TP53 mutant AML.

FIG. 12 shows increase in the abundance of CD80+ cells and/or CD80 expression in tumor following administration of SL-172154.

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DETAILED DESCRIPTION

The present disclosure is based, in part, on the discovery that CD80+ cells, CD8+ cells, Granzyme B+ cells, CD68+ cells, Ki67+ cells, and PD-L1+ immune cells increase in the tumor microenvironment (TME) following the administration of the SIRP α -Fc-CD40L chimeric protein, which is well-tolerated with no DLTs or evidence of anemia, thrombocytopenia, liver dysfunction, cytokine release syndrome or pneumonitis. The present disclosure is also based, in part, on the discoveries that B cells and/or CD40+ cells marginate from peripheral blood and the levels of innate and adaptive serum cytokines such as CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC increase within hours of following the administration of the SIRP α -Fc-CD40L chimeric protein, and that the B cells and/or CD40+ cells return to peripheral blood as well as the levels of innate and adaptive serum cytokines such as CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC decrease within about a day following the administration of the SIRP α -Fc-CD40L chimeric protein. In some embodiments, the levels of B cells and/or CD40+ cells, as well as the levels of innate and adaptive serum cytokines such as CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC get close to the background levels within a day or two of the administration of the SIRP α -Fc-CD40L chimeric protein.

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5 Importantly, since a chimeric protein of the present disclosure (*via* binding of the extracellular domain of CD172a (SIRP α) to its receptor/ligand on a cancer cell) disrupts, blocks, reduces, inhibits, and/or sequesters the transmission of immune inhibitory signals, *e.g.*, originating from a cancer cell that is attempting to avoid its phagocytosis and/or destruction, and (*via* binding of CD40L to its receptor) enhances, increases, and/or stimulates the transmission of an immune stimulatory signal to an anti-cancer immune cell, it can provide an
10 anti-tumor effect by two distinct pathways; this dual-action is more likely to provide any anti-tumor effect in a patient and/or to provide an enhanced anti-tumor effect in a patient. Furthermore, since such chimeric proteins can act *via* two distinct pathways, they can be efficacious, at least, in patients who respond poorly to treatments that target one of the two pathways. Thus, a patient who is a poor responder to treatments acting *via* one of the two pathway, can receive a therapeutic benefit by targeting the other pathway.

15 *Chimeric Proteins*

The chimeric proteins of the present disclosure comprise an extracellular domain of CD172a (SIRP α) and an extracellular domain of CD40L which together can simultaneously block immune inhibitory signals and stimulate immune activating signals.

Aspects of the present disclosure provide a chimeric protein comprising a general structure of: N terminus –
20 (a) – (b) – (c) – C terminus, where (a) is a first domain comprising an extracellular domain of CD172a (SIRP α), (b) is a linker adjoining the first domain and the second domain, *e.g.*, the linker comprising at least one cysteine residue capable of forming a disulfide bond and/or comprising a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of CD40L; wherein the linker connects the first domain and the second domain.

25 In embodiments, the first domain comprises substantially all of the extracellular domain of CD172a (SIRP α). In embodiments, the first domain is capable of binding a CD172a (SIRP α) ligand. In embodiments, the first domain is capable of binding a CD172a (SIRP α) ligand (*e.g.* CD47) expressed on cancer cell surface. In embodiments, the first domain is capable of inhibiting the binding of a CD172a (SIRP α) ligand (*e.g.* CD47) to the CD172a (SIRP α) protein located on myeloid and hematopoietic stem cells and neurons. In
30 embodiments, the first domain is capable of inhibiting an immunosuppressive signal. In embodiments, the first domain is capable of inhibiting an immunosuppressive signal. In embodiments, the first domain is capable of inhibiting a macrophage checkpoint or “do not eat me” signal. In embodiments the therapy with the SIRP α -Fc-CD40L chimeric protein (*e.g.* SEQ ID NO: 59 or SEQ ID NO: 61) stimulates macrophages to phagocytize tumor cells and effectively present the tumor antigens of phagocytized tumor cells to T cells.

5 In embodiments, the second domain is capable of binding a CD40 receptor. In embodiments, the second domain comprises substantially all of the extracellular domain of CD40L. In embodiments, the second domain is capable of activating an immune stimulatory signal.

In embodiments, the chimeric protein is a recombinant fusion protein, *e.g.*, a single polypeptide having the extracellular domains disclosed herein. For example, in embodiments, the chimeric protein is translated as a
10 single unit in a prokaryotic cell, a eukaryotic cell, or a cell-free expression system.

In embodiments, the present chimeric protein is producible in a mammalian host cell as a secretable and fully functional single polypeptide chain.

In embodiments, chimeric protein refers to a recombinant protein of multiple polypeptides, *e.g.*, multiple extracellular domains disclosed herein, that are combined (*via* covalent or non-covalent bonding) to yield a
15 single unit, *e.g.*, *in vitro* (*e.g.*, with one or more synthetic linkers disclosed herein).

In embodiments, the chimeric protein is chemically synthesized as one polypeptide or each domain is chemically synthesized separately and then combined. In embodiments, a portion of the chimeric protein is translated and a portion is chemically synthesized.

In embodiments, an extracellular domain refers to a portion of a transmembrane protein which is capable of
20 interacting with the extracellular environment. In embodiments, an extracellular domain refers to a portion of a transmembrane protein which is sufficient for binding to a ligand or receptor and is effective in transmitting a signal to a cell. In embodiments, an extracellular domain is the entire amino acid sequence of a transmembrane protein which is normally present at the exterior of a cell or of the cell membrane. In
25 embodiments, an extracellular domain is that portion of an amino acid sequence of a transmembrane protein which is external of a cell or of the cell membrane and is needed for signal transduction and/or ligand binding as may be assayed using methods known in the art (*e.g.*, *in vitro* ligand binding and/or cellular activation assays).

Transmembrane proteins typically consist of an extracellular domain, one or a series of transmembrane domains, and an intracellular domain. Without wishing to be bound by theory, the extracellular domain of a
30 transmembrane protein is responsible for interacting with a soluble receptor or ligand or membrane-bound receptor or ligand (*i.e.*, a membrane of an adjacent cell). Without wishing to be bound by theory, the transmembrane domain(s) is responsible for localizing the transmembrane protein to the plasma membrane. Without wishing to be bound by theory, the intracellular domain of a transmembrane protein is responsible

5 for coordinating interactions with cellular signaling molecules to coordinate intracellular responses with the extracellular environment (or visa-versa).

There are generally two types of single-pass transmembrane proteins: Type I transmembrane proteins which have an extracellular amino terminus and an intracellular carboxy terminus and Type II transmembrane proteins which have an extracellular carboxy terminus and an intracellular amino terminus. Type I and Type
10 II transmembrane proteins can be either receptors or ligands. For Type I transmembrane proteins (e.g., CD172a (SIRP α)), the amino terminus of the protein faces outside the cell, and therefore contains the functional domains that are responsible for interacting with other binding partners (either ligands or receptors) in the extracellular environment. For Type II transmembrane proteins (e.g., CD40L), the carboxy terminus of the protein faces outside the cell, and therefore contains the functional domains that are responsible for
15 interacting with other binding partners (either ligands or receptors) in the extracellular environment. Thus, these two types of transmembrane proteins have opposite orientations to each other relative to the cell membrane.

The description of CD47 as a “do not eat me” signal in a broad range of cancers stimulated exploration of what combinations of “eat me” signals may enhance antitumor immunity in the setting of CD47 blockade.
20 Willingham *et al.*, The CD47-signal regulatory protein alpha (SIRP α) interaction is a therapeutic target for human solid tumors. *Proc Natl Acad Sci U S A* 109: 6662-6667 (2012); Jaiswal *et al.*, CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* 138:271-285 (2009); Weiskopf *et al.*, Engineered SIRP α variants as immunotherapeutic adjuvants to anticancer antibodies. *Science* 341:88–91 (2013). Preclinical combinations of CD47 blockade and ADCP-competent antibodies,
25 including rituximab and trastuzumab, enhance tumor phagocytosis. Kauder *et al.*, ALX148 blocks CD47 and enhances innate and adaptive antitumor immunity with a favorable safety profile. *PLoS One* 13: e0201832 (2018); Chao *et al.*, Anti- CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. *Cell* 142:699–713 (2010); Chao *et al.*, Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. *Sci Transl Med* 2:63ra94 (2010); Advani
30 *et al.*, CD47 Blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma. *N Engl J Med* 379:1711-1721 (2018); Zhao *et al.*, CD47-signal regulatory protein-alpha (SIRP α) interactions form a barrier for antibody-mediated tumor cell destruction. *Proc Natl Acad Sci U S A* 108:18342-18347 (2011). At least 50% of patients with relapsed or refractory diffuse large B-cell lymphoma or follicular lymphoma treated with Hu5F9-G4, a humanized, IgG4 isotype, CD47-blocking mAb, in combination with rituximab demonstrate
35 objective responses. Advani *et al.*, CD47 Blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma.

- 5 N Engl J Med 379:1711-1721 (2018). CD47 blockade enhances antigen presentation in immune-neglected tumors (Tseng *et al.*, Anti-CD47 antibody-mediated phagocytosis of cancer by macrophages primes an effective antitumor T-cell response. Proc Natl Acad Sci U S A 110:11103-11108 (2013)), yet only sporadic clinical responses have been observed using CD47/SIRP α blocking therapeutics as monotherapy or in combination with PD-1/L1–blocking antibodies.
- 10 Disrupting the binding of CD47 to SIRP α has emerged as a promising immunotherapeutic strategy for advanced cancers by potentiating antibody-dependent cellular phagocytosis (ADCP) of targeted antibodies. Preclinically, CD47/SIRP α blockade induces antitumor activity by increasing the phagocytosis of tumor cells by macrophages and enhancing the cross-presentation of tumor antigens to CD8+ T cells by dendritic cells; both of these processes are potentiated by CD40 signaling. Here a novel, two-sided fusion protein
- 15 incorporating the extracellular domains of SIRP α and CD40L, adjoined by a central Fc domain, termed SIRP α -Fc-CD40L was generated. As shown herein, the SIRP α -Fc-CD40L chimeric protein bound CD47 and CD40 with high affinity and activated CD40 signaling in the absence of Fc receptor cross-linking. No evidence of hemolysis, hemagglutination, or thrombocytopenia was observed *in vitro* or in cynomolgus macaques. Further, as shown herein, the SIRP α -Fc-CD40L chimeric protein outperformed CD47 blocking and CD40
- 20 agonist antibodies in murine CT26 tumor models and synergized with immune checkpoint blockade of PD-1 and CTLA4. SIRP α -Fc-CD40L activated a type I interferon response in macrophages and potentiated the activity of ADCP-competent targeted antibodies both in vitro and in vivo. These data illustrated that whereas CD47/SIRP α inhibition could potentiate tumor cell phagocytosis, CD40-mediated activation of a type I interferon response provided a bridge between macrophage- and T-cell–mediated immunity that significantly
- 25 enhanced durable tumor control and rejection.

Chimeric proteins of the present disclosure comprise an extracellular domain of CD172a (SIRP α) and an extracellular domain of CD40L. Thus, a chimeric protein of the present disclosure comprises, at least, a first domain comprising the extracellular domain of CD172a (SIRP α), which is connected – directly or *via* a linker – to a second domain comprising the extracellular domain of CD40L. When the domains are linked in an

30 amino-terminal to carboxy-terminal orientation, the first domain is located on the “left” side of the chimeric protein and is “outward facing” and the second domain is located on “right” side of the chimeric protein and is “outward facing”.

Other configurations of first and second domains are envisioned, *e.g.*, the first domain is outward facing and the second domain is inward facing, the first domain is inward facing and the second domain is outward

- 5 facing, and the first and second domains are both inward facing. When both domains are “inward facing”, the chimeric protein would have an amino-terminal to carboxy-terminal configuration comprising an extracellular domain of CD40L, a linker, and an extracellular domain of CD172a (SIRP α). In such configurations, it may be necessary for the chimeric protein to include extra “slack”, as described elsewhere herein, to permit binding domains of the chimeric protein to one or both of its receptors/ligands.
- 10 Constructs could be produced by cloning of the nucleic acids encoding the three fragments (the extracellular domain of CD172a (SIRP α), followed by a linker sequence, followed by the extracellular domain of CD40L) into a vector (plasmid, viral or other) wherein the amino terminus of the complete sequence corresponded to the ‘left’ side of the molecule containing the extracellular domain of CD172a (SIRP α) and the carboxy terminus of the complete sequence corresponded to the ‘right’ side of the molecule containing the
- 15 extracellular domain of CD40L. In some embodiments of chimeric proteins having one of the other configurations, as described above, a construct would comprise three nucleic acids such that the translated chimeric protein produced would have the desired configuration, e.g., a dual inward-facing chimeric protein. Accordingly, In embodiments, the present chimeric proteins are engineered as such.

CD172a (SIRP α)-Fc-CD40L Chimeric Protein

- 20 In embodiments, the chimeric protein is capable of contemporaneously binding the human CD172a (SIRP α) ligand and the human CD40 receptor, wherein the CD172a (SIRP α) ligand is CD47 and the CD40L receptor is CD40.

The chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, in which (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In

25 embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond.

Chimeric proteins of the present disclosure have a first domain which is sterically capable of binding its ligand/receptor and/or a second domain which is sterically capable of binding its ligand/receptor. This means

30 that there is sufficient overall flexibility in the chimeric protein and/or physical distance between an extracellular domain (or portion thereof) and the rest of the chimeric protein such that the ligand/receptor binding domain of the extracellular domain is not sterically hindered from binding its ligand/receptor. This flexibility and/or physical distance (which is herein referred to as “slack”) may be normally present in the extracellular domain(s), normally present in the linker, and/or normally present in the chimeric protein (as a

5 whole). Alternately, or additionally, the chimeric protein may be modified by including one or more additional amino acid sequences (e.g., the joining linkers described below) or synthetic linkers (e.g., a polyethylene glycol (PEG) linker) which provide additional slack needed to avoid steric hindrance.

In embodiments, the chimeric proteins of the present disclosure comprise variants of the extracellular domain of CD172a (SIRP α). As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the known amino acid sequence of CD172a (SIRP α), e.g., human CD172a (SIRP α).

In embodiments, the extracellular domain of CD172a (SIRP α) has the following amino acid sequence:

20 EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLT
 KRNNMDFSIRIGNITPADAGTYCVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVWVGPAARATPQ
 HTVSFTCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTRDVDHSQVICEV
 AHVTLQGDPLRG TANLSETIRVPPTLEV TQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVS
 TETASTVTENKDGTYNWMWLLVNVSAHRDDVKLTQVEHDGQPAVSKSHDLKVS AHPKEQGSN
 25 TAAENTGSNERNIY (SEQ ID NO: 57).

In embodiments, a chimeric protein comprises a variant of the extracellular domain of CD172a (SIRP α). As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about

5 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 57.

In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 57. In
10 embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence
15 of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 57.

One of ordinary skill may select variants of the known amino acid sequence of CD172a (SIRP α) by consulting the literature, *e.g.* Hatherley *et al.*, "Paired receptor specificity explained by structures of signal regulatory proteins alone and complexed with CD47." *Mol Cell* 31: 266-277 (2008); Hatherley *et al.*, "The Structure of
20 the Macrophage Signal Regulatory Protein Alpha (Sirpalph α) Inhibitory Receptor Reveals a Binding Face Reminiscent of that Used by T Cell Receptors." *J Biol Chem* 282: 14567 (2007); Hatherley *et al.*, "Structure of Signal-Regulatory Protein Alpha: A Link to Antigen Receptor Evolution." *J Biol Chem* 284: 26613 (2009); Hatherley *et al.*, "Polymorphisms in the Human Inhibitory Signal-Regulatory Protein Alpha Do not Affect Binding to its Ligand Cd47." *J Biol Chem* 289: 10024 (2014); Ring *et al.*, "Anti-SIRP alpha antibody
25 immunotherapy enhances neutrophil and macrophage antitumor activity." *Proc Natl Acad Sci U S A* 114: E10578-E10585 (2017), each of which is incorporated by reference in its entirety.

In embodiments, the chimeric proteins of the present disclosure comprise variants of the extracellular domain of CD40L. As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least
30 about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at

- 5 least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the known amino acid sequence of CD40L, *e.g.*, human CD40L.

In embodiments, the extracellular domain of CD40L has the following amino acid sequence:

10 HRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEM
 QKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLENGKQLTVKRQGLYYIYAQVTFCS
 NREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQSQSIHLGGVFELQPGASVFNVTDPSPQ
 VSHGTGFTSFGLLKL (SEQ ID NO: 58).

- In embodiments, a chimeric protein comprises a variant of the extracellular domain of CD40L. As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%,
 15 or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%,
 20 or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 58.

- In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 58. In
 25 embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the
 30 second domain of a chimeric protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 58.

One of ordinary skill may select variants of the known amino acid sequence of CD40L by consulting the literature, *e.g.* Karpusas *et al.*, "2 A crystal structure of an extracellular fragment of human CD40 ligand."

5 Structure 3: 1031-1039 (1995); Karpusas *et al.*, "Structure of CD40 ligand in complex with the Fab fragment of a neutralizing humanized antibody." Structure 9: 321-329 (2001); Silvian *et al.*, "Small Molecule Inhibition of the TNF Family Cytokine CD40 Ligand through a Subunit Fracture Mechanism." ACS Chem Biol 6: 636-647 (2011); An *et al.*, "Crystallographic and mutational analysis of the CD40-CD154 complex and its implications for receptor activation." J Biol Chem 286: 11226-11235 (2011); Karnell *et al.*, "A CD40L-targeting protein reduces autoantibodies and improves disease activity in patients with autoimmunity." Sci Transl Med 10 11 (2019), each of which is incorporated by reference in its entirety.

In embodiments, the linker of a chimeric protein comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker of a chimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In 15 embodiments, the linker of a chimeric protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker of a chimeric protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker of a chimeric protein 20 comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker of a chimeric protein comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, a chimeric protein of the present disclosure comprises: (1) a first domain comprising the amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to SEQ ID NO: 25 57, (b) a second domain comprises the amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to SEQ ID NO: 58, and (c) a linker comprises an amino acid sequence that is that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

30 In embodiments, a chimeric protein of the present disclosure comprises: (1) a first domain comprising the amino acid sequence that is at least 95% identical to SEQ ID NO: 57, (b) a second domain comprises the amino acid sequence that is at least 95% identical to SEQ ID NO: 58, and (c) a linker comprises an amino acid sequence that is that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, a chimeric protein of the present disclosure comprises: (1) a first domain

5 comprising the amino acid sequence that is at least 97% identical to SEQ ID NO: 57, (b) a second domain comprises the amino acid sequence that is at least 97% identical to SEQ ID NO: 58, and (c) a linker comprises an amino acid sequence that is that is at least 97% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, a chimeric protein of the present disclosure comprises: (1) a first domain comprising the amino acid sequence that is at least 98% identical to SEQ ID NO: 57, (b) a
 10 second domain comprises the amino acid sequence that is at least 98% identical to SEQ ID NO: 58, and (c) a linker comprises an amino acid sequence that is that is at least 98% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, a chimeric protein of the present disclosure comprises: (1) a first domain comprising the amino acid sequence that is at least 99% identical to SEQ ID NO: 57, (b) a second domain comprises the amino acid sequence that is at least 99% identical to
 15 SEQ ID NO: 58, and (c) a linker comprises an amino acid sequence that is that is at least 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, a chimeric protein of the present disclosure comprises: (1) a first domain comprising the amino acid sequence of SEQ ID NO: 57, (b) a second domain comprises the amino acid sequence of SEQ ID NO: 58, and (c) a linker comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID
 20 NO: 3. In embodiments, a chimeric protein of the present disclosure comprises: (1) a first domain comprising the amino acid sequence identical to SEQ ID NO: 57, (b) a second domain comprises the amino acid sequence that is to SEQ ID NO: 58, and (c) a linker comprises an amino acid sequence that is that is identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, a CD172a (SIRP α)-Fc-CD40L chimeric protein of the present disclosure has the following
 25 amino acid sequence (the extracellular domain of CD172a (SIRP α) is shown in a boldface font, the extracellular domain of CD40L is indicated by underline, Fc domain is shown in italic:

EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSD
LTKRNNMDFSIRIGNITPADAGTYCYVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVVS GPAAR
ATPQHTVSFTCESHGFS PRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVL TREDVHS
 30 **QVICEVAHVTLQGDPLRG TANLSETIRVPPTLEV TQQPVR AENQVNVTCQVRKFYPQRLQLTWL**
ENGNVSR TETASTVTENKDGTYNWM SWLLVNVSAHRDDVKLT CQVEHDGQPAVSKSHDLKVS
AHPKEQGSNTAAENTGSNERNIYSKYGPPCPPCPA**PEFLGGPSVFLFPPKPKDQLMISRTPEVTC**
VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL**SGKEYKCKVS**
SKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 35 **NYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGKIEGRMDH**

5 RRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEM
 QKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLENGKQLTVKRQGLYYIYAQVTFC
 SNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGASVFNVTDP
 SQVSHGTGFTSFGLLKL (SEQ ID NO: 59).

10 The 792 amino acid sequence of the CD172a (SIRP α)-Fc-CD40L chimeric protein (SL-172154) (not including the leader sequence) is shown above. The CD172a (SIRP α)-Fc-CD40L chimeric protein exists as a profile of oligomeric forms. There are 17 cysteines in the amino acid sequence with 8 likely disulfide pairs. Both N and O-linked glycosylation have been identified.

15 In embodiments, the chimeric protein of the present disclosure comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 potential N glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least two potential N glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least four potential N glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least six potential N glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least eight potential N glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least ten potential N glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least 1, 2, 3, 4, 5, 6, 7, or 8 potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least two potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least four potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least six potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least eight potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least two potential N glycosylation sites, and at least two potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least four potential N glycosylation sites, and at least four potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least six potential N glycosylation sites, and at least six potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least eight potential N glycosylation sites, and at least eight potential O glycosylation sites. In embodiments, the chimeric protein of the present disclosure comprises at least ten potential N glycosylation sites, and at least eight potential O glycosylation sites. In embodiments, the chimeric protein expressed in Chinese Hamster Ovary (CHO) cells is glycosylated.

5 There are 17 cysteines present in the SL-172154 chimeric protein. In some embodiments, the SL-172154 chimeric protein has no disulfide bonds. In some embodiments, the SL-172154 chimeric protein has at least one, or at least two, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8, or at least 9, or at least 10 disulfide bonds. In some embodiments, the SL-172154 chimeric protein has at least one, or at least two interchain disulfide bonds. In some embodiments, the SL-172154 chimeric protein has at least one, or at least two, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or 8 intrachain disulfide bonds. In some embodiments, the SL-172154 chimeric protein has a C350=C350 interchain disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C353=C353 interchain disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C153=C153 interchain disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C25 = C91 disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C140 = C198 disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C243 = C301 disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C385 = C445 disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C491 = C549 disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C603 = C615 disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C709 = C725 disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C140 = C243 = C709/C725 scrambled disulfide bond. In some embodiments, the SL-172154 chimeric protein has a C615 (chain1) = C615 (chain2) scrambled disulfide bond.

In some embodiments, the CD172a (SIRP α)-Fc-CD40L chimeric protein of the present disclosure is encoded by the following nucleotide sequence (leader sequence is shown by a bold-underlined font):

25 **ATGGAATGGAGCTGGGTGTTCTTCTTCTTCTGTCGTCGACACCGGCGTGCACTCG**GAGGAGGAG
CTCCAGGTCATCCAGCCGACAAAGTCGGTGCTCGTGGCCGCCGAGAACTGCCACCCTGAGGTGC
ACCGCGACCTCGCTGATTCCCGTGGGCCCGATTCAAGTGGTCCGGGGGGCCGGGCCTGGCAGAGAA
CTGATCTACAACCAGAAGGAAGGCCATTTCCCTCGCGTGACTACTGTGTCCGATCTTACTAAGCGGAA
CAACATGGACTTCAGCATTAGGATCGGCAACATCACCCCTGCTGACGCGGGAACCTACTACTGCGTCA
30 AGTTCAGGAAAGGAAGCCCGGACGACGTGGAGTTCAAGAGCGGGGCGGGCACCGAACTGTCCGTGC
GCGCCAAGCCATCCGCGCCCGTGGTGTCCGGACCCGACCCAGAGCAACTCCGCAGCACACCGTGT
CGTTCACCTTGCGAATCACACGGATTCTCCCCGCGGATATCACGCTTAAGTGGTTCAAGAACGGGAAC
GAACTGAGCGACTTCCAGACCAACGTGGACCCCGTCGGAGAAAGCGTCAGCTACTCCATTCACTCGA
CCGCCAAAGTGGTGCTGACCAAGGAGGACGTGCATAGCCAAGTGATCTGCGAGGTCGCCACGTC
35 CTCTGCAAGGAGATCCGCTGCGGGGAACAGCCAACCTGTCCGAACTATCCGCGTGCCTCCACCCCT

5 GGAAGTGACCCAGCAGCCCGTCCGAGCGGAGAATCAAGTCAATGTGACCTGTCAAGTCCGGAAATTC
TACCCTCAACGGCTCCAGCTGACCTGGCTGGAAAACGGAAACGTGTCCCGCACGGAAACCGCCTCGA
CCGTGACCGAGAACAAGGACGGCACCTACAACCTGGATGTCCTGGCTCTTGGTGAACGTGTCAGCCCA
CCGGGACGATGTCAAGCTGACTTGCCAAGTGGAAACATGATGGGCAGCCAGCTGTCAGCAAGAGCCAC
GACCTGAAGGTGTCCGCGCACCCGAAGGAACAGGGTTCGAATACTGCCGCCGAAAACTGGTAGCA
10 ACGAACGGAACATCTACTCTAAGTACGGCCACCTTGCCCTCCCTGCCCGGCACCTGAATTTCTGGGT
GGACCCTCCGTGTTTCTTTTCCCGCCCAAGCCAAAGGACCAGTTGATGATCTCCCGCACTCCGGAAGT
GACATGCGTGGTGGTGGACGTGTCCAGGAAGATCCGGAAGTGCAGTTCAATTGGTACGTGGATGGC
GTGGAGGTCCATAACGCCAAGACTAAGCCGCGCGAGGAACAGTTCAATTCCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCATCAGGACTGGCTCTCCGGCAAAGAGTACAAGTGAAGGTGTCATCCAA
15 GGGTCTGCCGTGTCATCGAAAAGACCATTTCGAATGCCACTGGGCAGCCCAGAGAACCTCAAGTCT
ACACCCTCCCACCGTCCCAAGAGGAAATGACCAAGAACCAAGTCTCGCTGACGTGTCTCGTGAAGGG
ATTCTACCCATCCGACATTGCTGTGGAATGGGAGTCCAACGGCCAGCCCGAGAACAACCTACAAGACTA
CCCCTCCCGTCCCTGGACTCCGACGGTTCTTCTTCTTCTTACTCTCGCCTCACCGTGGATAAGTCGCGG
TGGCAGGAGGGGAACGTGTTCTCCTGCTCCGTCTGCACGAAGCATTGCACAACCACTACACCCAGA
20 AGTCCCTGTCACTGTCCCTGGGAAAGATTGAGGGTCGGATGGATCATCGGCGCCTGGACAAGATCGA
GGACGAGCGGAACCTCCACGAGGATTTCTGTTCATGAAAACCATCCAGAGATGCAACACCGGAGAG
AGAAGCCTGTCCCTGCTCAACTGCGAGGAAATCAAGTCCCAGTTTGAAGGATTTGTGAAGGACATTAT
GCTGAACAAGGAAGAGACTAAGAAGGAAAACTCCTTCGAGATGCAGAAGGGCGATCAGAACCCACAG
ATCGCGGCCACGTGATCTCCGAGGCCTCGTCAAAGACCACTTCAGTGCTCCAATGGGCCGAGAAGG
25 GTTACTATACCATGAGCAACAACCTTGTGACCCTGGAGAACGGAAAGCAGCTCACCGTGAAAAGACAG
GGACTGTACTATATCTATGCCCAAGTCACCTTCTGTTTGAACCGCGAGGCTAGCAGCCAGGCCCGTT
CATCGCCTCCCTCTGTTTGAAGTCGCCGGGGCGGTTTGAAGGATTCTGCTGAGAGCTGCGAATACC
CATTTCGTCCGCCAAGCCTTGCGGACAGCAGTCAATCCACCTGGGGGGAGTGTTGAGCTGCAGCCTG
GCGCGAGCGTGTTGTCGAACGTGACCGACCCCTCCCAAGTGTCTCACGGCACCGGATTCACTTCGTT
30 TGGCCTGCTGAAGCTGTAA (SEQ ID NO: 60)

In some embodiments, the SEQ ID NO: 60 encodes for a precursor of the CD172a (SIRP α)-Fc-CD40L chimeric protein of the present disclosure having following amino acid sequence (leader sequence is shown by an italic font):

MEWSWVFLFSLVTTGVHSEEELQVIQPKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQK
35 EGHFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYCYVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVVS

5 GPAARATPQHTVSFTCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTRDVDHSQ
 VICEVAHVTLQGDPLRG TANLSETIRVPPTLEV TQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSR T
 ETASTVTENKDGTYNWM SWLLVNVSAHRDDVKLT CQVEHDGQPAVSKSHDLKVS AHPKEQGSNTAAENT
 GSNERNIYSKYGPPCPPCPAPEFLGGPSVFLFPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG
 VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTSNATGQPREPQVYTL P
 10 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNV
 FSCSVLHEALHNHYTQKSLSLGLKIEGRMDHRR LDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEI
 KSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLENG
 KQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFEL
 QPGASVFVNVTDP SQVSHGTGFTSFGLLKL (SEQ ID NO: 61)

15 The chimeric protein of SEQ ID NO: 59 (also referred to herein as SL-172154) is a recombinant fusion glycoprotein comprising the extracellular domain of human CD172a (SIRPα) (PDCD1, CD272a), a central domain including the hinge-CH2-CH3 region from human immunoglobulin constant gamma 4 (Inhibitory receptor SHPS-1, IgG4), and the extracellular domain of human CD40L (TNFSF5, TRAP, CD154). The linear configuration of SL-172154 is CD172a (SIRPα)-Fc-CD40L.

20 The predicted molecular weight for the monomeric chimeric protein of SEQ ID NO: 59 is 88.1 kDa. The predicted molecular weight for the glycosylated monomeric chimeric protein of SEQ ID NO: 59 is about 115 kDa.

The dual-sided nature of the chimeric proteins disclosed herein, such as the CD172a (SIRPα)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61), is designed to intercept one of the key immunosuppressive pathways within the tumor microenvironment (TME): the CD172a (SIRPα) – CD47 macrophage checkpoint.

Tumor cells may express CD47 on their cell surface, which can bind to CD172a (SIRPα) expressed by a macrophage to suppress phagocytosis of the tumor cells. Thus, the CD172a (SIRPα)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) can bind to CD47 expressed on the surface of tumor, with the CD172a (SIRPα) domain of the CD172a (SIRPα)-Fc-CD40L chimeric proteins disclosed herein intended to provide competitive inhibition of CD47, and to replace the CD47 inhibitory signal with functionally trimerized/hexamerized CD40L, resulting in an incoming T cell experiencing co-stimulation *via* engagement through its CD40 receptor instead of suppression through CD172a (SIRPα) interactions. In other words, because the extracellular domains (ECDs) of CD172a (SIRPα) and CD40L are physically linked to one

5 another and localized to the TME, tumor infiltrating T cells will receive co-stimulation at the same time they recognize a tumor antigen *via* its T cell receptor (TCR). Importantly, because the ECDs of CD172a (SIRP α) and CD40L are physically linked to one another, and localized to the TME, tumor infiltrating T cells will receive costimulation at the same time they recognize a tumor antigen *via* the T cell receptor. Together, these would result in replacement of an inhibitory CD47 signal with a co-stimulatory CD40L signal to enhance the anti-tumor activity of T cells.

The three constituent components of the chimeric proteins disclosed herein, including the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61), have unique attributes that facilitate dimerization or oligomerization. The extracellular domain of CD172a (SIRP α) normally exists as a monomer and is not known to form higher-order homomeric complexes. The central Fc domain contains cysteine residues that are capable of disulfide bonding to form a dimeric structure. In embodiments, the chimeric proteins disclosed herein, including the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61), contains an S228P mutation in the hinge region of the Fc domain to prevent Fab arm exchange. The CD40L domain is known to form homotrimeric complexes, which are stabilized through noncovalent, electrostatic interactions. Although the chimeric proteins disclosed herein, including the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61), are expressed as a continuous monomeric protein by production cell lines, the resulting monomeric proteins self-assemble into higher-order species based on these disulfide and charge-based interactions of CD40L (creating a trimer) and the combined influence of these attractive forces, resulting in a hexamer (dimer of trimers). The majority (>80%) of the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) comprises the hexamer and trimer forms, which have similar activity. Importantly, because the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61), are comprised of hexamers and trimers, they stimulate CD40 signaling in the absence of cross-linking by Fc receptors or any other cross-linking agent. The predicted tertiary structures of the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) as a monomer and in various oligomeric states, based on disulfide (Fc) and charge-based (CD40L) interactions shows visualization by electron microscopy of the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) hexamers (top two images) and the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) trimers (bottom two images). Accordingly, the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) forms trimers/hexamers and activates CD40 without the need for cross-linking. It is noteworthy that, unlike

- 5 monoclonal antibodies, Fc receptor cross-linking is not required for functional activity of the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61).

In embodiments, a chimeric protein comprises a variant of the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61). As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%,
10 or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%,
15 or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or at least about 99.2%, or at least about 99.4%, or at least about 99.6%, or at least about 99.8% sequence identity with SEQ ID NO: 59 or SEQ ID NO: 61.

In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least
20 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the first domain
25 of a chimeric protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61.

- 30 In embodiments, the first domain is capable of binding a CD172a (SIRP α) ligand.

In embodiments, the first domain comprises substantially all of the extracellular domain of CD172a (SIRP α).

In embodiments, the second domain is capable of binding a CD40 receptor.

In embodiments, the second domain comprises substantially all of the extracellular domain of CD40L.

5 In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, e.g., human IgG4.

In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the first domain comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain of a chimeric protein comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 57.

In embodiments, the second domain comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least

5 97% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the second domain of a chimeric protein comprises an amino acid sequence that is identical to the amino acid
10 sequence of SEQ ID NO: 58.

In embodiments, the (a) the first domain comprises the amino acid sequence of SEQ ID NO: 57, (b) the second domain comprises the amino acid sequence of SEQ ID NO: 58, and (c) the linker comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the chimeric protein further comprises an amino acid sequence that is at least 90%, or 93%,
15 or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 5 and/or SEQ ID NO: 7. In embodiments, the chimeric protein further comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 5 and/or SEQ ID NO: 7. In embodiments, the chimeric protein further comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 5 and/or SEQ ID NO: 7. In embodiments, the chimeric protein further comprises the amino
20 acid sequence of SEQ ID NO: 5 and/or SEQ ID NO: 7.

In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 95% identical to SEQ ID NO: 59 or SEQ ID NO: 61, *e.g.*, at least about 98% identical to SEQ ID NO: 59 or SEQ ID NO: 61, at least about 99% identical to SEQ ID NO: 59 or SEQ ID NO: 61, at least about 99.2% identical to SEQ ID NO: 59 or SEQ ID NO: 61, at least about 99.4% identical to SEQ ID NO: 59 or SEQ ID NO: 61, at least about
25 99.6% identical to SEQ ID NO: 59 or SEQ ID NO: 61, or at least about 99.8% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61.

In any herein-disclosed aspect and embodiment, the chimeric protein may comprise an amino acid sequence having one or more amino acid mutations relative to any of the protein sequences disclosed herein. In
30 embodiments, the one or more amino acid mutations may be independently selected from substitutions, insertions, deletions, and truncations.

In embodiments, the amino acid mutations are amino acid substitutions, and may include conservative and/or non-conservative substitutions. "Conservative substitutions" may be made, for instance, on the basis of similarity in polarity, charge, size, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of

- 5 the amino acid residues involved. The 20 naturally occurring amino acids can be grouped into the following six standard amino acid groups: (1) hydrophobic: Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr; Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. As used herein, "conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed within the same group of the six standard amino acid groups shown above. For example, the exchange of Asp by Glu retains one negative charge in the so modified polypeptide. In addition, glycine and proline may be substituted for one another based on their ability to disrupt α -helices. As used herein, "non-conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed in a different group of the six standard amino acid groups (1) to (6) shown above.
- 10
- 15 In embodiments, the substitutions may also include non-classical amino acids (e.g., selenocysteine, pyrrolysine, *N*-formylmethionine β -alanine, GABA and δ -Aminolevulinic acid, 4-aminobenzoic acid (PABA), D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogs in general).
- 20

Mutations may also be made to the nucleotide sequences of the chimeric proteins by reference to the genetic code, including taking into account codon degeneracy.

- 25 In embodiments, a chimeric protein is capable of binding human ligand(s)/receptor(s).

In embodiments, each extracellular domain (or variant thereof) of the chimeric protein binds to its cognate receptor or ligand with a K_D of about 1 nM to about 5 nM, for example, about 1 nM, about 1.5 nM, about 2 nM, about 2.5 nM, about 3 nM, about 3.5 nM, about 4 nM, about 4.5 nM, or about 5 nM. In embodiments, the chimeric protein binds to a cognate receptor or ligand with a K_D of about 5 nM to about 15 nM, for example,

30 about 5 nM, about 5.5 nM, about 6 nM, about 6.5 nM, about 7 nM, about 7.5 nM, about 8 nM, about 8.5 nM, about 9 nM, about 9.5 nM, about 10 nM, about 10.5 nM, about 11 nM, about 11.5 nM, about 12 nM, about 12.5 nM, about 13 nM, about 13.5 nM, about 14 nM, about 14.5 nM, or about 15 nM.

In embodiments, each extracellular domain (or variant thereof) of the chimeric protein binds to its cognate receptor or ligand with a K_D of less than about 1 μ M, about 900 nM, about 800 nM, about 700 nM, about 600

5 nM, about 500 nM, about 400 nM, about 300 nM, about 200 nM, about 150 nM, about 130 nM, about 100 nM, about 90 nM, about 80 nM, about 70 nM, about 60 nM, about 55 nM, about 50 nM, about 45 nM, about 40 nM, about 35 nM, about 30 nM, about 25 nM, about 20 nM, about 15 nM, about 10 nM, or about 5 nM, or about 1 nM (as measured, for example, by surface plasmon resonance or biolayer interferometry).

In embodiments, the chimeric protein binds to human CD47 with a K_D of about 1 nM to about 5 nM, for
10 example, about 1 nM, about 1.5 nM, about 2 nM, about 2.5 nM, about 3 nM, about 3.5 nM, about 4 nM, about 4.5 nM, or about 5 nM. In embodiments, the chimeric protein binds to human CD47 with a K_D of less than about 3 nM, about 2 nM, about 1 nM, about 900 pM, about 800 pM, about 700 pM, about 600 pM, about 500 pM, about 400 pM, about 300 pM, about 200 pM, about 100 pM, about 90 pM, about 80 pM, about 70 pM, about 60 pM about 55 pM about 50 pM about 45 pM, about 40 pM, about 35 pM, about 30 pM, about 25 pM,
15 about 20 pM, about 15 pM, or about 10 pM, or about 1 pM (as measured, for example, by surface plasmon resonance or biolayer interferometry).

In embodiments, the chimeric protein binds to human CD40 with a K_D of less than about 1 nM, about 900 pM, about 800 pM, about 700 pM, about 600 pM, about 500 pM, about 400 pM, about 300 pM, about 200 pM, about 100 pM, about 90 pM, about 80 pM, about 70 pM, about 60 pM about 55 pM about 50 pM about
20 45 pM, about 40 pM, about 35 pM, about 30 pM, about 25 pM, about 20 pM, about 15 pM, or about 10 pM, or about 1 pM (as measured, for example, by surface plasmon resonance or biolayer interferometry).

As used herein, a variant of an extracellular domain is capable of binding the receptor/ligand of a native extracellular domain. For example, a variant may include one or more mutations in an extracellular domain which do not affect its binding affinity to its receptor/ligand; alternately, the one or more mutations in an
25 extracellular domain may improve binding affinity for the receptor/ligand; or the one or more mutations in an extracellular domain may reduce binding affinity for the receptor/ligand, yet not eliminate binding altogether. In embodiments, the one or more mutations are located outside the binding pocket where the extracellular domain interacts with its receptor/ligand. In embodiments, the one or more mutations are located inside the binding pocket where the extracellular domain interacts with its receptor/ligand, as long as the mutations do
30 not eliminate binding altogether. Based on the skilled artisan's knowledge and the knowledge in the art regarding receptor-ligand binding, s/he would know which mutations would permit binding and which would eliminate binding.

In embodiments, the chimeric protein exhibits enhanced stability and protein half-life.

5 A chimeric protein of the present disclosure may comprise more than two extracellular domains. For example, the chimeric protein may comprise three, four, five, six, seven, eight, nine, ten, or more extracellular domains. A second extracellular domain may be separated from a third extracellular domain *via* a linker, as disclosed herein. Alternately, a second extracellular domain may be directly linked (*e.g.*, *via* a peptide bond) to a third extracellular domain. In embodiments, a chimeric protein includes extracellular domains that are directly
10 linked and extracellular domains that are indirectly linked *via* a linker, as disclosed herein.

Linkers

In embodiments, the chimeric protein comprises a linker.

In embodiments, the linker comprising at least one cysteine residue capable of forming a disulfide bond. The at least one cysteine residue is capable of forming a disulfide bond between a pair (or more) of chimeric
15 proteins. Without wishing to be bound by theory, such disulfide bond forming is responsible for maintaining a useful multimeric state of chimeric proteins. This allows for efficient production of the chimeric proteins; it allows for desired activity *in vitro* and *in vivo*.

In a chimeric protein of the present disclosure, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, or an antibody sequence.

20 In embodiments, the linker is derived from naturally-occurring multi-domain proteins or is an empirical linker as described, for example, in Chichili *et al.*, *Protein Sci.* 22(2):153-167 (2013); Chen *et al.*, *Adv Drug Deliv Rev.* 65(10):1357-1369 (2013), the entire contents of which are hereby incorporated by reference. In embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen *et al.*, *Adv Drug Deliv Rev.* 65(10):1357-1369 (2013); and Crasto *et al.*, *Protein Eng.*
25 13(5):309-312 (2000), the entire contents of which are hereby incorporated by reference.

In embodiments, the linker comprises a polypeptide. In embodiments, the polypeptide is less than about 500 amino acids long, about 450 amino acids long, about 400 amino acids long, about 350 amino acids long, about 300 amino acids long, about 250 amino acids long, about 200 amino acids long, about 150 amino acids long, or about 100 amino acids long. For example, the linker may be less than about 100, about 95, about
30 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids long.

5 In embodiments, the linker is flexible.

In embodiments, the linker is rigid.

In embodiments, the linker is substantially comprised of glycine and serine residues (e.g., about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 97%, or about 98%, or about 99%, or about 100% glycines and serines).

10 In embodiments, the linker comprises a hinge region of an antibody (e.g., of IgG, IgA, IgD, and IgE, inclusive of subclasses (e.g., IgG1, IgG2, IgG3, and IgG4, and IgA1, and IgA2)). The hinge region, found in IgG, IgA, IgD, and IgE class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses. For example, the length and flexibility of the hinge
15 region varies among the IgG subclasses. The hinge region of IgG1 encompasses amino acids 216-231 and, because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, is relatively short, and contains a rigid poly-proline double helix, stabilized by extra inter-heavy chain disulfide
20 bridges. These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), containing 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3, the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge
25 region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge regions reportedly decreases in the order IgG3>IgG1>IgG4>IgG2. In embodiments, the linker may be derived from human IgG4 and contain one or more mutations to enhance dimerization (including S228P) or FcRn binding.

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally
30 into three regions: the upper hinge region, the core region, and the lower hinge region. See Shin *et al.*, *Immunological Reviews* 130:87 (1992). The upper hinge region includes amino acids from the carboxyl end of C_{H1} to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide

5 bridges, and the lower hinge region joins the amino terminal end of the C_{H2} domain and includes residues in C_{H2}. *Id.* The core hinge region of wild-type human IgG1 contains the sequence CPPC (SEQ ID NO: 24) which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. In embodiments, the present linker comprises, one, or two, or three of the upper hinge region, the core region, and the lower hinge region of any antibody (e.g., of IgG, IgA, IgD, and IgE, inclusive
10 of subclasses (e.g., IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)). The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains five glycosylation sites within a 17-amino-acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin. In embodiments, the linker of the present disclosure
15 comprises one or more glycosylation sites.

In embodiments, the linker comprises an Fc domain of an antibody (e.g., of IgG, IgA, IgD, and IgE, inclusive of subclasses (e.g., IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)).

In a chimeric protein of the present disclosure, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from a human IgG4.
20 In embodiments, the linker comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 1 to SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of any one of SEQ ID NO: 1 to SEQ ID NO: 3 (e.g., at least 95% identical to the amino acid sequence of SEQ ID NO: 2.). In embodiments, the linker comprises one or more joining linkers, such joining
25 linkers independently selected from SEQ ID NOs: 4-50 (or a variant thereof). In embodiments, the linker comprises two or more joining linkers each joining linker independently selected from SEQ ID NOs: 4-50 (or a variant thereof); 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.

In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from a human IgG1 antibody. In
30 embodiments, the Fc domain exhibits increased affinity for and enhanced binding to the neonatal Fc receptor (FcRn). In embodiments, the Fc domain includes one or more mutations that increases the affinity and enhances binding to FcRn. Without wishing to be bound by theory, it is believed that increased affinity and enhanced binding to FcRn increases the *in vivo* half-life of the present chimeric proteins.

5 In embodiments, the Fc domain in a linker contains one or more amino acid substitutions at amino acid residue 250, 252, 254, 256, 308, 309, 311, 416, 428, 433, or 434 (in accordance with Kabat numbering, as in as in Kabat, *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) expressly incorporated herein by reference), or equivalents thereof. In embodiments, the amino acid substitution at amino acid residue 250 is a substitution with
10 glutamine. In embodiments, the amino acid substitution at amino acid residue 252 is a substitution with tyrosine, phenylalanine, tryptophan or threonine. In embodiments, the amino acid substitution at amino acid residue 254 is a substitution with threonine. In embodiments, the amino acid substitution at amino acid residue 256 is a substitution with serine, arginine, glutamine, glutamic acid, aspartic acid, or threonine. In embodiments, the amino acid substitution at amino acid residue 308 is a substitution with threonine. In
15 embodiments, the amino acid substitution at amino acid residue 309 is a substitution with proline. In embodiments, the amino acid substitution at amino acid residue 311 is a substitution with serine. In embodiments, the amino acid substitution at amino acid residue 385 is a substitution with arginine, aspartic acid, serine, threonine, histidine, lysine, alanine or glycine. In embodiments, the amino acid substitution at amino acid residue 386 is a substitution with threonine, proline, aspartic acid, serine, lysine, arginine,
20 isoleucine, or methionine. In embodiments, the amino acid substitution at amino acid residue 387 is a substitution with arginine, proline, histidine, serine, threonine, or alanine. In embodiments, the amino acid substitution at amino acid residue 389 is a substitution with proline, serine or asparagine. In embodiments, the amino acid substitution at amino acid residue 416 is a substitution with serine. In embodiments, the amino acid substitution at amino acid residue 428 is a substitution with leucine. In embodiments, the amino acid substitution at amino acid residue 433 is a substitution with arginine, serine, isoleucine, proline, or glutamine.
25 In embodiments, the amino acid substitution at amino acid residue 434 is a substitution with histidine, phenylalanine, or tyrosine.

In embodiments, the Fc domain linker (*e.g.*, comprising an IgG constant region) comprises one or more mutations such as substitutions at amino acid residue 252, 254, 256, 433, 434, or 436 (in accordance with
30 Kabat numbering, as in as in Kabat, *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) expressly incorporated herein by reference). In embodiments, the IgG constant region includes a triple M252Y/S254T/T256E mutation or YTE mutation. In embodiments, the IgG constant region includes a triple H433K/N434F/Y436H mutation or KFH mutation. In embodiments, the IgG constant region includes an YTE and KFH mutation in combination.

5 In embodiments, the linker comprises an IgG constant region that contains one or more mutations at amino acid residues 250, 253, 307, 310, 380, 428, 433, 434, and 435 (in accordance with Kabat numbering, as in as in Kabat, *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) expressly incorporated herein by reference). Illustrative mutations include T250Q, M428L, T307A, E380A, I253A, H310A, M428L, H433K, N434A, N434F, N434S, and H435A.

10 In embodiments, the IgG constant region comprises a M428L/N434S mutation or LS mutation. In embodiments, the IgG constant region comprises a T250Q/M428L mutation or QL mutation. In embodiments, the IgG constant region comprises an N434A mutation. In embodiments, the IgG constant region comprises a T307A/E380A/N434A mutation or AAA mutation. In embodiments, the IgG constant region comprises an I253A/H310A/H435A mutation or IHH mutation. In embodiments, the IgG constant region comprises a

15 H433K/N434F mutation. In embodiments, the IgG constant region comprises a M252Y/S254T/T256E and a H433K/N434F mutation in combination.

Additional exemplary mutations in the IgG constant region are described, for example, in Robbie, *et al.*, Antimicrobial Agents and Chemotherapy 57(12):6147-6153 (2013); Dall'Acqua *et al.*, *Journal Biol Chem* 281(33):23514-24 (2006); Dall'Acqua *et al.*, *Journal of Immunology* 169:5171-80 (2002); Ko *et al.* *Nature* 20 514:642-645 (2014); Grevys *et al.* *Journal of Immunology* 194(11):5497-508 (2015); and U.S. Patent No. 7,083,784, the entire contents of which are hereby incorporated by reference.

An illustrative Fc stabilizing mutant is S228P. Illustrative Fc half-life extending mutants are T250Q, M428L, V308T, L309P, and Q311S and the present linkers may comprise 1, or 2, or 3, or 4, or 5 of these mutants.

In embodiments, the chimeric protein binds to FcRn with high affinity. In embodiments, the chimeric protein may bind to FcRn with a K_D of about 1 nM to about 80 nM. For example, the chimeric protein may bind to FcRn with a K_D of about 1 nM, about 2 nM, about 3 nM, about 4 nM, about 5 nM, about 6 nM, about 7 nM, about 8 nM, about 9 nM, about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM, about 50 nM, about 55 nM, about 60 nM, about 65 nM, about 70 nM, about 71 nM, about 72 nM, about 73 nM, about 74 nM, about 75 nM, about 76 nM, about 77 nM, about 78 nM, about 25 79 nM, or about 80 nM. In embodiments, the chimeric protein may bind to FcRn with a K_D of about 9 nM. In embodiments, the chimeric protein does not substantially bind to other Fc receptors (*i.e.* other than FcRn) with effector function.

In embodiments, the Fc domain in a linker has the amino acid sequence of SEQ ID NO: 1 (see **Table 1**, below), or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto. In embodiments, mutations

- 5 are made to SEQ ID NO: 1 to increase stability and/or half-life. For instance, in embodiments, the Fc domain in a linker comprises the amino acid sequence of SEQ ID NO: 2 (see **Table 1**, below), or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto. For instance, in embodiments, the Fc domain in a linker comprises the amino acid sequence of SEQ ID NO: 3 (see **Table 1**, below), or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto.
- 10 Further, one or more joining linkers may be employed to connect an Fc domain in a linker (e.g., one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto) and the extracellular domains. For example, any one of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or variants thereof may connect an extracellular domain as disclosed herein and an Fc domain in a linker as disclosed herein. Optionally, any one of SEQ ID NOs: 4 to
- 15 50, or variants thereof are located between an extracellular domain as disclosed herein and an Fc domain as disclosed herein.

In embodiments, the present chimeric proteins may comprise variants of the joining linkers disclosed in **Table 1**, below. For instance, a linker may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about

20 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least

25 about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 4 to 50.

In embodiments, the first and second joining linkers may be different or they may be the same.

Without wishing to be bound by theory, including a linker comprising at least a part of an Fc domain in a

30 chimeric protein, helps avoid formation of insoluble and, likely, non-functional protein concatemers and/or aggregates. This is in part due to the presence of cysteines in the Fc domain which are capable of forming disulfide bonds between chimeric proteins.

In embodiments, a chimeric protein may comprise one or more joining linkers, as disclosed herein, and lack a Fc domain linker, as disclosed herein.

- 5 In embodiments, the first and/or second joining linkers are independently selected from the amino acid sequences of SEQ ID NOs: 4 to 50 and are provided in **Table 1** below:

Table 1: Illustrative linkers (Fc domain linkers and joining linkers)

SEQ ID NO.	Sequence
1	APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLT VDKSSWQEGNVFSCSVMEALHNHYTQKSLSLGLGK
2	APEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTPHSDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLT VDKSSWQEGNVFSCSVLHEALHNHYTQKSLSLGLGK
3	APEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLT VDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLGLGK
4	SKYGPPCPSCP
5	SKYGPPCPPCP
6	SKYGPP
7	IEGRMD
8	GGGVPRDCG
9	IEGRMDGGGGAGGGG
10	GGGSGGGG
11	GGGSGGGSGGGG
12	EGKSSGSGSESKST
13	GGSG
14	GGSGGGSGGGSG
15	EAAAKEAAAKEAAAK
16	EAAAREAAAREAAAREAAAR

17	GGGSGGGSGGGGSAS
18	GGGGAGGGG
19	GS or GGS or LE
20	GS GSGS
21	GSGSGSGSGS
22	GGGGSAS
23	APAPAPAPAPAPAPAPAP
24	CPPC
25	GGGGS
26	GGGSGGGGS
27	GGGSGGGSGGGGS
28	GGGSGGGSGGGSGGGGS
29	GGGSGGGSGGGSGGGSGGGGS
30	GGGSGGGSGGGSGGGSGGGSGGGGS
31	GGGSGGGSGGGSGGGSGGGSGGGSGGGGS
32	GGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGS
33	GGSGGSGGGSGGGGS
34	GGGGGGGG
35	GGGGGG
36	EAAAK
37	EAAAKEAAK
38	EAAAKEAAKEAAK
39	AEAAAKEAAKA
40	AEAAAKEAAKEAAKA
41	AEAAAKEAAKEAAKEAAKA
42	AEAAAKEAAKEAAKEAAKEAAKA
43	AEAAAKEAAKEAAKEAAKALEAAAKEAAKEAAKEAAKA
44	PAPAP
45	KESGSVSSEQLAQFRSLD
46	GSAGSAAGSGEF

5

TABLE 2: Illustrative modular linkers

Joining Linker 1	Fc	Joining Linker 2	Modular Linker = Joining Linker 1 + Fc + Joining Linker 2
SKYGPPCPSCP (SEQ ID NO: 4)	APEFLGGPSVFLFPPKPKDTL MISRTPEVTCVWVDVSQEDPE VQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDW LSGKEYKCKVSSKGLPSSIEKT ISNATGQPREPQVYTLPPSQUE EMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTPPVL DSDGSFFLYSRLTVDKSSWQE GNVFSCSVMHHEALHNHYTQK LSLSLGLK (SEQ ID NO: 1)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPSCPAPPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVWVDV SQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQ DWLSGKEYKCKVSSKGLPSSIEK TISNATGQPREPQVYTLPPSQUE MTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDG SFFLYSRLTVDKSSWQEGNVFSC SVMHHEALHNHYTQKLSLSLGLKIE GRMD (SEQ ID NO: 51)
SKYGPPCPSCP (SEQ ID NO: 4)	APEFLGGPSVFLFPPKPKDQL MISRTPEVTCVWVDVSQEDPE VQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTTPHSDW LSGKEYKCKVSSKGLPSSIEKT ISNATGQPREPQVYTLPPSQUE EMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTPPVL DSDGSFFLYSRLTVDKSSWQE GNVFSCSVLHEALHNHYTQKS LSLSLGLK (SEQ ID NO: 2)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPSCPAPPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVWVD VSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTTPH SDWLSGKEYKCKVSSKGLPSSIE KTISNATGQPREPQVYTLPPSQUE EMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDG SFFLYSRLTVDKSSWQEGNVFSC SVLHEALHNHYTQKLSLSLGLKIE GRMD (SEQ ID NO: 52)
SKYGPPCPSCP (SEQ ID NO: 4)	APEFLGGPSVFLFPPKPKDQL MISRTPEVTCVWVDVSQEDPE VQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDW LSGKEYKCKVSSKGLPSSIEKT	IEGRMD (SEQ ID NO: 7)	SKYGPPCPSCPAPPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVWVD VSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLH QDWLSGKEYKCKVSSKGLPSSIE

	ISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTPPVL DSDGSFFLYSRLTVDKSRWQE GNVFSCSVLHEALHNHYTQKS LSLSLGK (SEQ ID NO: 3)		KTISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSC SVLHEALHNHYTQKSLSLSLGKIE GRMD (SEQ ID NO: 53)
SKYGPPCPPCP (SEQ ID NO: 5)	APEFLGGPSVFLFPPKPKDTL MISRTPEVTCVWVDVSQEDPE VQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDW LSGKEYKCKVSSKGLPSSIEKT ISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTPPVL DSDGSFFLYSRLTVDKSSWQE GNVFSCSVLHEALHNHYTQK SLLSLGK (SEQ ID NO: 1)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVWVDV SQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQ DWLSGKEYKCKVSSKGLPSSIEK TISNATGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDG SFFLYSRLTVDKSSWQEGNVFSC SVLHEALHNHYTQKSLSLSLGKIE GRMD (SEQ ID NO: 54)
SKYGPPCPPCP (SEQ ID NO: 5)	APEFLGGPSVFLFPPKPKDQL MISRTPEVTCVWVDVSQEDPE VQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTPHSDW LSGKEYKCKVSSKGLPSSIEKT ISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTPPVL DSDGSFFLYSRLTVDKSSWQE GNVFSCSVLHEALHNHYTQKS LSLSLGK (SEQ ID NO: 2)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPPCPAPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVWVD VSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTPH SDWLSGKEYKCKVSSKGLPSSIE KTISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDG SFFLYSRLTVDKSSWQEGNVFSC SVLHEALHNHYTQKSLSLSLGKIE GRMD (SEQ ID NO: 55)
SKYGPPCPPCP (SEQ ID NO: 5)	APEFLGGPSVFLFPPKPKDQL MISRTPEVTCVWVDVSQEDPE VQFNWYVDGVEVHNAKTKPR	IEGRMD (SEQ ID NO: 7)	SKYGPPCPPCPAPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVWVD VSQEDPEVQFNWYVDGVEVHNA

EEQFNSTYRVVSVLTVLHQDW LSGKEYKCKVSSKGLPSSIEKT ISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTPPVVL DSDGSFFLYSRLTVDKSRWQE GNVFSCSVLHEALHNHYTQKS LSLSLGK (SEQ ID NO: 3)	KTKPREEQFNSTYRVVSVLTVLH QDWLSGKEYKCKVSSKGLPSSIE KTISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSC SVLHEALHNHYTQKSLSLGLKIE GRMD (SEQ ID NO: 56)
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- 5 In embodiments, the present chimeric proteins may comprise variants of the modular linkers disclosed in **Table 2**, above. For instance, a linker may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%,
10 or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity
15 with the amino acid sequence of any one of SEQ ID NOs: 51 to 56.

In embodiments, the linker may be flexible, including without limitation highly flexible. In embodiments, the linker may be rigid, including without limitation a rigid alpha helix. Characteristics of illustrative joining linkers is shown below in **Table 3**:

TABLE 3: Characteristics of illustrative joining linkers

Joining Linker Sequence	Characteristics
SKYGPPCPPCP (SEQ ID NO: 5)	IgG4 Hinge Region
IEGRMD (SEQ ID NO: 7)	Linker
GGGVPRDCG (SEQ ID NO: 8)	Flexible
GGGSGGGS (SEQ ID NO: 10)	Flexible
GGGSGGGGSGGG (SEQ ID NO: 11)	Flexible

Joining Linker Sequence	Characteristics
EGKSSGSGSESKST (SEQ ID NO: 12)	Flexible + soluble
GGSG (SEQ ID NO: 13)	Flexible
GGSGGGSGGGSG (SEQ ID NO: 14)	Flexible
EAAAKEAAAKEAAK (SEQ ID NO: 15)	Rigid Alpha Helix
EAAAREAAAREAAAREAAAR (SEQ ID NO: 16)	Rigid Alpha Helix
GGGGSGGGSGGGGSAS (SEQ ID NO: 17)	Flexible
GGGGAGGGG (SEQ ID NO: 18)	Flexible
GS (SEQ ID NO: 19)	Highly flexible
GSGSGS (SEQ ID NO: 20)	Highly flexible
GSGSGSGSGS (SEQ ID NO: 21)	Highly flexible
GGGGSAS (SEQ ID NO: 22)	Flexible
APAPAPAPAPAPAPAPAP (SEQ ID NO: 23)	Rigid

- 5 In embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the present chimeric protein. In another example, the linker may function to target the chimeric protein to a particular cell type or location.

In embodiments, a chimeric protein comprises only one joining linkers.

- 10 In embodiments, a chimeric protein lacks joining linkers.

In embodiments, the linker is a synthetic linker such as polyethylene glycol (PEG).

- 15 In embodiments, a chimeric protein has a first domain which is sterically capable of binding its ligand/receptor and/or the second domain which is sterically capable of binding its ligand/receptor. Thus, there is enough overall flexibility in the chimeric protein and/or physical distance between an extracellular domain (or portion thereof) and the rest of the chimeric protein such that the ligand/receptor binding domain of the extracellular domain is not sterically hindered from binding its ligand/receptor. This flexibility and/or physical distance (which is referred to as "slack") may be normally present in the extracellular domain(s), normally present in the linker, and/or normally present in the chimeric protein (as a whole). Alternately, or additionally, an amino acid sequence (for example) may be added to one or more extracellular domains and/or to the linker to
- 20 provide the slack needed to avoid steric hindrance. Any amino acid sequence that provides slack may be

- 5 added. In embodiments, the added amino acid sequence comprises the sequence (Gly)_n where n is any number from 1 to 100. Additional examples of addable amino acid sequence include the joining linkers described in **Table 1** and **Table 3**. In embodiments, a polyethylene glycol (PEG) linker may be added between an extracellular domain and a linker to provide the slack needed to avoid steric hindrance. Such PEG linkers are well known in the art.
- 10 In embodiments, a chimeric protein of the present disclosure comprises the extracellular domain of human CD172a (SIRPα) (or a variant thereof), a linker, and the extracellular domain of human CD40L (or a variant thereof). In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain, e.g., from an IgG1 or from IgG4, including human IgG1 or IgG4. Thus, in embodiments, a chimeric protein of the present disclosure comprises the extracellular domain of human CD172a (SIRPα) (or a variant thereof), linker comprising a
- 15 hinge-CH2-CH3 Fc domain, and the extracellular domain of human CD40L (or a variant thereof). Such a chimeric protein may be referred to herein as “hCD172a (SIRPα)-Fc-CD40L” or “SL-172154”.

Diseases, Methods of Treatment, and Mechanisms of Action

- An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises (i) administering to the human subject a first dose of chimeric protein having a general
- 20 structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRPα)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level and/or activity of a cell has been measured in a biological sample obtained from the subject before the administration of the
- 25 first dose, wherein the marker is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell. In embodiments, the method further comprises (ii) administering to the human subject a second dose of the chimeric protein if a post-dosing level and/or activity of the cell is greater than the background level and/or activity of the cell. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose.
- 30 In embodiments, the biological sample is selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the biological sample

5 is a biopsy sample or a surgical specimen. In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the a tumor biopsy sample derived from a tumor selected ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN).

In embodiments, the level and/or activity of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell
10 sorting (FACS) or a combination thereof. In embodiments, the level and/or activity of the cell is measured by contacting the sample with an agent that specifically binds to one or more molecules selected from CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a recombinant antibody,
15 a monoclonal antibody, a polyclonal antibody, or fragment thereof.

In embodiments, the level and/or activity of the cell is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding one or more of CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

20 An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of chimeric protein having a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising
25 an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level and/or activity of B cells and/or CD40+ cells has been measured in a first biological sample obtained from the subject before the administration of the first dose. In embodiments, an N-hr post-dose level and/or activity of B cells and/or CD40+ cells has been measured in a second biological sample obtained from the subject after the administration of the first dose. In embodiments, N is a number between 1 and 24. In embodiments, the N-
30 hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, an M-day post-dose level and/or activity of B cells and/or CD40+ cells has been measured in a third biological sample obtained from the subject after the administration of the first dose. In embodiments, M is a number between 1 and 28. In embodiments, the method further comprises (ii) administering to the human subject a second dose of the chimeric protein if the

5 M-day post-dose level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose.

In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1. In embodiments, M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2. In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.

In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is greater than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is within about 10%, or about 20%, or about 30%, or about 40% of the background level and/or activity of B cells and/or CD40+ cells.

In embodiments, the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood.

In embodiments, level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof. In embodiments, level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to a CD40 and/or a B-cell marker. In embodiments, the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof. In embodiments, level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that

5 specifically binds to one or more nucleic acids encoding a CD40 and/or a B-cell marker. In embodiments, the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background amount and/or activity of a cytokine selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC has been measured in a first biological sample obtained from the subject before the administration of the first dose. In embodiments, a N-hr post-dose amount and/or activity of the cytokine has been measured in a second biological sample obtained from the subject after the administration of the first dose, In embodiments, N is a number between 1 and 24. In embodiments, the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine. In embodiments, the M-day post-dose amount and/or activity of the cytokine has been measured in a third biological sample obtained from the subject after the administration of the first dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises (ii) administering to the human subject a second dose of the chimeric protein if the M-day post-dose amount and/or activity of the cytokine is at least about 30% lower than the N-hr post-dose amount and/or activity of the cytokine. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose.

An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level of a cell has been measured in a pre-dose biological sample obtained from the subject before the administration of the first dose, wherein the marker is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell. In embodiments, the method further comprises

5 administering to the human subject a second dose of the chimeric protein if a post-dosing level of the cell is greater than the background level of the cell. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose.

In embodiments, the biological sample is selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating
10 nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the biological sample is a biopsy sample or a surgical specimen. In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the tumor biopsy sample or the tumor surgical specimen
15 derived from a tumor selected ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN).

In embodiments, the level and/or activity of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof. In embodiments, the level and/or activity of the cell is measured by
20 contacting the sample with an agent that specifically binds to one or more molecules selected from CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof. In embodiments, the level and/or activity of the cell is measured by contacting the sample with an agent that specifically binds to one or more nucleic
25 acids encoding one or more of CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular
30 domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level and/or activity of B cells and/or CD40+ cells has been measured in a first biological sample obtained from the subject before the administration of the first dose. In embodiments, an N-hr post-dose level and/or activity of B cells and/or

5 CD40+ cells has been measured in a second biological sample obtained from the subject after the administration of the first dose. In embodiments, N is a number between 1 and 24. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, an M-day post-dose level and/or activity of B cells and/or CD40+ cells has been measured in a third biological sample obtained from the subject after the
 10 administration of the first dose. In embodiments, M is a number between 1 and 28. In embodiments, the method further comprises (ii) administering to the human subject a second dose of the chimeric protein if the M-day post-dose level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose.

15 In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1. In embodiments, M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2. In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.

20 In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is greater than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is within about 10%, or about 20%, or about 30%, or about 40% of the background level and/or activity of B cells
 25 and/or CD40+ cells.

In embodiments, the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal
 30 swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood.

In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and

5 fluorescent activating cell sorting (FACS) or a combination thereof. In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to a CD40 and/or a B-cell marker. In embodiments, the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a
10 recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof. In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding a CD40 and/or a B-cell marker. In embodiments, the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic
15 acid primer or probe.

An aspect of the present disclosure is a method for treating a cancer in a human subject. In embodiments, the method comprises: (i) administering to the human subject a first dose of the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second
20 domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background amount and/or activity of a cytokine selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC has been measured in a first biological sample obtained from the subject before the administration of the first dose. In embodiments, a N-hr post-dose amount and/or activity of the cytokine has
25 been measured in a second biological sample obtained from the subject after the administration of the first dose. In embodiments, N is a number between 1 and 24. In embodiments, the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine. In embodiments, the M-day post-dose amount and/or activity of the cytokine has been measured in a third biological sample obtained from the subject after the administration of the first dose. In embodiments, M is a number between
30 1 and 28. In embodiments, the method further comprises (ii) administering to the human subject a second dose of the chimeric protein if the M-day post-dose amount and/or activity of the cytokine is at least about 30% lower than the N-hr post-dose amount and/or activity of the cytokine. In embodiments, the second dose is administered at least about 48 hours after the administration of the first dose.

In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than
35 2, or less than 1. In embodiments, M is less than 21, or less than 14, or less than 12, or less than 10, or less

5 than 8, or less than 6, or less than 4, or less than 2. In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.

In embodiments, the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine. In embodiments, the N-hr post-dose amount and/or activity of the cytokine is less than the background amount and/or activity of the cytokine. In embodiments, the N-hr post-dose amount and/or activity of the cytokine is within about 10%, or about 20%, or about 30%, or about 40% of the background amount and/or activity of the cytokine.

In embodiments, the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood.

20 In embodiments, the amount and/or activity of the cytokine is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof. In embodiments, the amount and/or activity of the cytokine is measured by contacting the sample with an agent that specifically binds to the cytokine. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof. In embodiments, the amount and/or activity of the cytokine is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding the cytokine. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

30 The chimeric proteins disclosed herein, including the CD172a (SIRPα)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61), finds use in methods for treating both advanced solid tumors and advanced lymphomas. These tumor types include: melanoma, non-small cell lung cancer (squamous, adeno, adeno-squamous), urothelial cancer, renal cell cancer, squamous cell cervical cancer, gastric or gastro-esophageal junction adenocarcinoma, squamous cell carcinoma of the anus, squamous cell carcinoma of the head and

5 neck, squamous cell carcinoma of the skin, and microsatellite instability high or mismatch repair deficient solid tumors excluding central nervous system (CNS) tumors. Other tumors of interest include Hodgkin's lymphoma (HL), diffuse large B cell lymphoma, acute myeloid leukemia (AML) and high-risk myelodysplastic syndromes (HR-MDS).

In embodiments, the cancer comprises an advanced solid tumor (local and/or metastatic). In embodiments, 10 the human subject has a cancer, wherein the cancer being treated is characterized by having macrophages in the tumor microenvironment and/or having tumor cells that are CD47+ cells in the tumor. In embodiments, the administration of the SIRP α -Fc-CD40L chimeric protein blocks the "don't eat me" signal of a tumor cell and /or stimulates an "eat me" signal. In embodiments the therapy with the SIRP α -Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) stimulates macrophages to phagocytize tumor cells and effectively 15 present the tumor antigens of phagocytized tumor cells to T cells.

In embodiments, the cancer is a solid cancer. In embodiments, the cancer is a solid tumor. In embodiments, the cancer is a metastatic cancer. In embodiments, the cancer is a hematological cancer. In embodiments, the cancer expresses CD47.

In embodiments, the cancer comprises an advanced lymphoma. In embodiments, the cancer comprises 20 acute myeloid leukemia (AML). In embodiments, the cancer comprises p53 mutant AML. In embodiments, the cancer comprises a high-risk myelodysplastic syndrome (HR-MDS).

Aspects of the present disclosure provide methods of treating cancer. The methods comprise a step of administering to a subject in need thereof an effective amount of a chimeric protein, e.g., in a pharmaceutical composition, as disclosed herein.

25 It is often desirable to enhance immune stimulatory signal transmission to boost an immune response, for instance to enhance a patient's anti-tumor immune response.

In embodiments, the chimeric protein of the present disclosure comprises an extracellular domain of human CD172a (SIRP α), which disrupts, blocks, reduces, inhibits, and/or sequesters the transmission of immune inhibitory signals, e.g., originating from a cancer cell that is attempting to avoid its detection and/or 30 destruction, and an extracellular domain of human CD40L, which enhances, increases, and/or stimulates the transmission of an immune stimulatory signal to an anti-cancer immune cell. Thus, the simultaneous binding of the extracellular domain of CD172a (SIRP α) to its ligand/receptor and the binding of the extracellular domain of CD40L with its receptor will prevent the transmission of an immunosuppressive signal from the

5 cancer cell and will have stimulate immune activity in an immune system cell. In other words, chimeric proteins of the present disclosure are capable of treating cancer *via* two distinct mechanisms.

In embodiments, the present disclosure pertains to cancers and/or tumors; for example, the treatment or prevention of cancers and/or tumors. As disclosed elsewhere herein, the treatment of cancer involves, in
10 embodiments, modulating the immune system with the present chimeric proteins to favor of increasing or activating immune stimulatory signals. In embodiments, the method reduces the amount or activity of regulatory T cells (Tregs) as compared to untreated subjects or subjects treated with antibodies directed to CD172a (SIRP α), CD40L, and/or their respective ligands or receptors. In embodiments, the method increases priming of effector T cells in draining lymph nodes of the subject as compared to untreated subjects
15 or subjects treated with antibodies directed to CD172a (SIRP α), CD40L, and/or their respective ligands or receptors. In embodiments, 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 treated with antibodies directed to the CD172a (SIRP α), CD40L, and/or their respective ligands or receptors.

In embodiments, the present chimeric proteins are capable of, or can be used in methods comprising, modulating the amplitude of an immune response, *e.g.* modulating the level of effector output. In
20 embodiments, *e.g.* when used for the treatment of cancer, the present chimeric proteins alter the extent of immune stimulation as compared to immune inhibition to increase the amplitude of a T cell response, including, without limitation, stimulating increased levels of cytokine production, proliferation or target killing potential. In embodiments, the patient's T cells are activated and/or stimulated by the chimeric protein, with the activated T cells being capable of dividing and/or secreting cytokines.

25 Cancers or tumors refer to an uncontrolled growth of cells and/or abnormal increased cell survival and/or inhibition of apoptosis which interferes with the normal functioning of the bodily organs and systems. Included are benign and malignant cancers, polyps, hyperplasia, as well as dormant tumors or micrometastases. Also, included are cells having abnormal proliferation that is not impeded by the immune system (*e.g.*, virus infected cells). The cancer may be a primary cancer or a metastatic cancer. The primary cancer may be an area of
30 cancer cells at an originating site that becomes clinically detectable, and may be a primary tumor. In contrast, the metastatic cancer may be the spread of a disease from one organ or part to another non-adjacent organ or part. The metastatic cancer may be caused by a cancer cell that acquires the ability to penetrate and infiltrate surrounding normal tissues in a local area, forming a new tumor, which may be a local metastasis. The cancer may also be caused by a cancer cell that acquires the ability to penetrate the walls of lymphatic

5 and/or blood vessels, after which the cancer cell is able to circulate through the bloodstream (thereby being a circulating tumor cell) to other sites and tissues in the body. The cancer may be due to a process such as lymphatic or hematogenous spread. The cancer may also be caused by a tumor cell that comes to rest at another site, re-penetrates through the vessel or walls, continues to multiply, and eventually forms another clinically detectable tumor. The cancer may be this new tumor, which may be a metastatic (or secondary)
10 tumor.

The cancer may be caused by tumor cells that have metastasized, which may be a secondary or metastatic tumor. The cells of the tumor may be like those in the original tumor. As an example, if a breast cancer or colon cancer metastasizes to the liver, the secondary tumor, while present in the liver, is made up of abnormal breast or colon cells, not of abnormal liver cells. The tumor in the liver may thus be a metastatic breast cancer
15 or a metastatic colon cancer, not liver cancer.

The cancer may have an origin from any tissue. The cancer may originate from melanoma, colon, breast, or prostate, and thus may be made up of cells that were originally skin, colon, breast, or prostate, respectively. The cancer may also be a hematological malignancy, which may be leukemia or lymphoma. The cancer may invade a tissue such as liver, lung, bladder, or intestinal.

20 In embodiments, the chimeric protein is used to treat a subject that has a treatment-refractory cancer. In embodiments, the chimeric protein is used to treat a subject that is refractory to one or more immune-modulating agents. For example, in embodiments, the chimeric protein is used to treat a subject that presents no response to treatment, or whose disease progresses, after 12 weeks or so of treatment. For instance, in embodiments, the subject is refractory to one or more CD172a (SIRPα) and/or CD47 agents, including, for
25 example, Magrolimab (5F9), Hu5F9-G4, CC-90002, Ti-061, SRF231, TTI-621, TTI-622, or ALX148 refractory patients. For instance, in embodiments, the subject is refractory to an anti-CTLA-4 agent, *e.g.*, ipilimumab (YERVOY)-refractory patients (*e.g.*, melanoma patients). Accordingly, in embodiments the present disclosure provides methods of cancer treatment that rescue patients that are non-responsive to various therapies, including monotherapy of one or more immune-modulating agents.

30 In embodiments, the present disclosure provides chimeric proteins which target a cell or tissue within the tumor microenvironment. In embodiments, the cell or tissue within the tumor microenvironment expresses one or more targets or binding partners of the chimeric protein. The tumor microenvironment refers to the cellular milieu, including cells, secreted proteins, physiological small molecules, and blood vessels in which the tumor exists. In embodiments, the cells or tissue within the tumor microenvironment are one or more of:

5 tumor vasculature; tumor-infiltrating lymphocytes; fibroblast reticular cells; endothelial progenitor cells (EPC); cancer-associated fibroblasts; pericytes; other stromal cells; components of the extracellular matrix (ECM); dendritic cells; antigen presenting cells; T-cells; regulatory T cells; macrophages; neutrophils; and other immune cells located proximal to a tumor. In embodiments, the present chimeric protein targets a cancer cell. In embodiments, the cancer cell expresses one or more of targets or binding partners of the chimeric protein.

10 The activation of regulatory T cells is critically influenced by costimulatory and co-inhibitory signals. Two major families of costimulatory molecules include the B7 and the tumor necrosis factor (TNF) families. These molecules bind to receptors on T cells belonging to the CD28 or TNF receptor families, respectively. Many well-defined co-inhibitors and their receptors belong to the B7 and CD28 families.

In embodiments, an immune stimulatory signal refers to a signal that enhances an immune response. For example, in the context of oncology, such signals may enhance antitumor immunity. For instance, without limitation, immune stimulatory signal may be identified by directly stimulating proliferation, cytokine production, killing activity, or phagocytic activity of leukocytes. For example, a chimeric protein may directly stimulate the proliferation and cytokine production of individual T cell subsets. Another example includes direct stimulation of an immune inhibitory cell with through a receptor that inhibits the activity of such an immune suppressor cell. This would include, for example, stimulation of CD4+FoxP3+ regulatory T cells, which would reduce the ability of those regulatory T cells to suppress the proliferation of conventional CD4+ or CD8+ T cells. In another example, this would include stimulation of CD40 on the surface of an antigen presenting cell, causing activation of antigen presenting cells including enhanced ability of those cells to present antigen in the context of appropriate native costimulatory molecules, including those in the B7 or TNF superfamily. In another example, the chimeric protein causes activation of the lymphoid cell and/or production of pro-inflammatory cytokines or chemokines to further stimulate an immune response, optionally within a tumor.

In embodiments, the present chimeric proteins are capable of, or find use in methods involving, enhancing, restoring, promoting and/or stimulating immune modulation. In embodiments, the present chimeric proteins described herein, restore, promote and/or stimulate the activity or activation of one or more immune cells against tumor cells including, but not limited to: 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. In embodiments, the present chimeric proteins enhance, restore, promote and/or stimulate the activity and/or activation of T cells, including, by way of a non-limiting example, activating and/or stimulating one or

5 more T- cell intrinsic signals, including a pro-survival signal; an autocrine or paracrine growth signal; a p38 MAPK-, ERK-, STAT-, JAK-, AKT- or PI3K-mediated signal; an anti-apoptotic signal; and/or a signal promoting and/or necessary for one or more of: pro-inflammatory cytokine production or T cell migration or T cell tumor infiltration.

In embodiments, the present chimeric proteins are capable of, or find use in methods involving, causing an
10 increase of one or more of T cells (including without limitation cytotoxic T lymphocytes, T helper cells, natural killer T (NKT) cells), B cells, natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells, monocytes, and macrophages (e.g., one or more of M1 and M2) into a tumor or the tumor microenvironment. In embodiments, the chimeric protein enhances recognition of tumor antigens by CD8+ T cells, particularly those T cells that have infiltrated into the tumor microenvironment. In embodiments, the present chimeric
15 protein induces CD19 expression and/or increases the number of CD19 positive cells (e.g., CD19 positive B cells). In embodiments, the present chimeric protein induces IL-15R α expression and/or increases the number of IL-15R α positive cells (e.g., IL-15R α positive dendritic cells).

In embodiments, the present chimeric proteins are capable of, or find use in methods involving, inhibiting and/or causing a decrease in immunosuppressive cells (e.g., myeloid-derived suppressor cells (MDSCs),
20 regulatory T cells (Tregs), tumor associated neutrophils (TANs), M2 macrophages, and tumor associated macrophages (TAMs)), and particularly within the tumor and/or tumor microenvironment (TME). In embodiments, the present therapies may alter the ratio of M1 versus M2 macrophages in the tumor site and/or TME to favor M1 macrophages. In embodiments, the SIRP α - Fc-CD40L chimeric protein suppresses/reduces/eliminates a “don’t eat me” signal via Sitr1a/CD47 from being transmitted on tumor
25 cells. In embodiments, the SIRP α - Fc-CD40L chimeric protein makes a tumor more likely to be attacked by the immune system of the subject. In embodiments, the SIRP α - Fc-CD40L chimeric protein makes a tumor more likely to be attacked by the innate immune system of the subject. In embodiments, the SIRP α - Fc-CD40L chimeric protein makes a tumor more likely to be attacked by the adaptive immune system of the subject. S In embodiments, the SIRP α - Fc-CD40L chimeric protein can suppress/reduce/eliminate binding of
30 tumor-overexpressed CD47 with phagocyte-expressed SIRP α to permit phagocytic removal of cancer cells and/or immunogenic processing of tumor antigens by macrophages and/or dendritic cells. In embodiments, the administration of the SIRP α - Fc-CD40L chimeric protein blocks the “don’t eat me” signal of a tumor cell and /or stimulates an “eat me” signal. In embodiments the therapy with the SIRP α -Fc-CD40L chimeric protein

- 5 (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) stimulates macrophages to phagocytize tumor cells and effectively present the tumor antigens of phagocytized tumor cells to T cells.

In embodiments, the present chimeric proteins are able to increase the serum levels of various cytokines including, but not limited to, one or more of IFN γ , TNF α , IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17A, IL-17F, and IL-22. In embodiments, the present chimeric proteins are capable of enhancing IL-2, IL-4, IL-5, IL-10, IL-13, IL-17A, IL-22, or IFN γ in the serum of a treated subject. In embodiments, the present chimeric proteins do not increase the serum levels of certain cytokines. In embodiments, the present chimeric proteins do not increase the serum levels of IL-6 and/ or TNF α . In embodiments, the present chimeric proteins do not increase the serum levels of IL-6 and/ or TNF α in the serum of a treated subject. In embodiments, the present chimeric proteins do not increase the serum levels of IL-6 and/ or TNF α in the serum of a treated subject, while increasing the levels of other cytokines, including but not limited to, CCL2, IL-8 and CXCL9 in serum of a treated subject. Detection of such a cytokine response may provide a method to determine the optimal dosing regimen for the indicated chimeric protein.

In a chimeric protein of the present disclosure, the chimeric protein is capable of increasing or preventing a decrease in a sub-population of CD4+ and/or CD8+ T cells.

- 20 In a chimeric protein of the present disclosure, the chimeric protein is capable of enhancing tumor killing activity by T cells.

In embodiments, the chimeric protein activates the human subject's T cells when bound by the CD40L 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 CD40 agonist antibodies and/or CD47 blocking antibodies.

- 25 In embodiments, the present chimeric proteins inhibit, block and/or reduce cell death of an anti-tumor CD8+ and/or CD4+ T cell; or stimulate, induce, and/or increase cell death of a pro-tumor T cell. T cell exhaustion is a state of T cell dysfunction characterized by progressive loss of proliferative and effector functions, culminating in clonal deletion. Accordingly, a pro-tumor T cell refers to a state of T cell dysfunction that arises during many chronic infections, inflammatory diseases, and cancer. This dysfunction is defined by poor proliferative and/or effector functions, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection
- 30

- 5 and tumors. Illustrative pro-tumor T cells include, but are not limited to, Tregs, CD4⁺ and/or CD8⁺ T cells expressing one or more checkpoint inhibitory receptors, Th2 cells and Th17 cells. Checkpoint inhibitory receptors refer to receptors expressed on immune cells that prevent or inhibit uncontrolled immune responses. In contrast, an anti-tumor CD8⁺ and/or CD4⁺ T cell refers to T cells that can mount an immune response to a tumor.
- 10 In embodiments, the present chimeric proteins are capable of, and can be used in methods comprising, increasing a ratio of effector T cells to regulatory T cells. Illustrative effector T cells include ICOS⁺ effector T cells; cytotoxic T cells (*e.g.*, αβ TCR, CD3⁺, CD8⁺, CD45RO⁺); CD4⁺ effector T cells (*e.g.*, αβ TCR, CD3⁺, CD4⁺, CCR7⁺, CD62L^{hi}, IL-7R/CD127⁺); CD8⁺ effector T cells (*e.g.*, αβ TCR, CD3⁺, CD8⁺, CCR7⁺, CD62L^{hi}, IL-7R/CD127⁺); effector memory T cells (*e.g.*, CD62L^{low}, CD44⁺, TCR, CD3⁺, IL-7R/CD127⁺, IL-15R⁺, CCR7^{low}); central memory T cells (*e.g.*, CCR7⁺, CD62L⁺, CD27⁺; or CCR7^{hi}, CD44⁺, CD62L^{hi}, TCR, CD3⁺, IL-7R/CD127⁺, IL-15R⁺); CD62L⁺ effector T cells; CD8⁺ effector memory T cells (TEM) including early effector memory T cells (CD27⁺ CD62L⁻) and late effector memory T cells (CD27⁻ CD62L⁻) (TemE and TemL, respectively); CD127⁽⁺⁾CD25^(low/-) effector T cells; CD127⁽⁻⁾CD25⁽⁻⁾ effector T cells; CD8⁺ stem cell memory effector cells (TSCM) (*e.g.*, CD44^(low)CD62L^(high)CD122^(high)sca⁽⁺⁾); TH1 effector T-cells (*e.g.*, CXCR3⁺, CXCR6⁺ and CCR5⁺; or αβ TCR, CD3⁺, CD4⁺, IL-12R⁺, IFNγR⁺, CXCR3⁺); TH2 effector T cells (*e.g.*, CCR3⁺, CCR4⁺ and CCR8⁺; or αβ TCR, CD3⁺, CD4⁺, IL-4R⁺, IL-33R⁺, CCR4⁺, IL-17RB⁺, CRTH2⁺); TH9 effector T cells (*e.g.*, αβ TCR, CD3⁺, CD4⁺); TH17 effector T cells (*e.g.*, αβ TCR, CD3⁺, CD4⁺, IL-23R⁺, CCR6⁺, IL-1R⁺); CD4⁺CD45RO⁺CCR7⁺ effector T cells, CD4⁺CD45RO⁺CCR7⁽⁻⁾ effector T cells; and effector T cells secreting IL-2, IL-4 and/or IFN-γ. Illustrative regulatory T cells include ICOS⁺ regulatory T cells, CD4⁺CD25⁺FOXP3⁺ regulatory T cells, CD4⁺CD25⁺ regulatory T cells, CD4⁺CD25⁻ regulatory T cells, CD4⁺CD25^{high} regulatory T cells, TIM-3⁺CD172a (SIRPα)⁺ regulatory T cells, lymphocyte activation gene-3 (LAG-3)⁺ regulatory T cells, CTLA-4/CD152⁺ regulatory T cells, neuropilin-1 (Nrp-1)⁺ regulatory T cells, CCR4⁺CCR8⁺ regulatory T cells, CD62L (L-selectin)⁺ regulatory T cells, CD45RBlow regulatory T cells, CD127^{low} regulatory T cells, LRRC32/GARP⁺ regulatory T cells, CD39⁺ regulatory T cells, GITR⁺ regulatory T cells, LAP⁺ regulatory T cells, 1B11⁺ regulatory T cells, BTLA⁺ regulatory T cells, type 1 regulatory T cells (Tr1 cells), T helper type 3 (Th3) cells, regulatory cell of natural killer T cell phenotype (NKTregs), CD8⁺ regulatory T cells, CD8⁺CD28⁻ regulatory T cells and/or regulatory T-cells secreting IL-10, IL-35, TGF-β, TNF-α, Galectin-1, IFN-γ and/or MCP1.
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In embodiments, the chimeric protein of the invention causes an increase in effector T cells (*e.g.*, CD4⁺CD25⁺ T cells).

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5 In embodiments, the chimeric protein causes a decrease in regulatory T cells (*e.g.*, CD4+CD25+ T cells).

In embodiments, the chimeric protein generates a memory response which may, *e.g.*, be capable of preventing relapse or protecting the animal from a recurrence and/or preventing, or reducing the likelihood of, metastasis. Thus, an animal treated with the chimeric protein is later able to attack tumor cells and/or prevent development of tumors when rechallenged after an initial treatment with the chimeric protein.

10 Accordingly, a chimeric protein of the present disclosure stimulates both active tumor destruction and also immune recognition of tumor antigens, which are essential in programming a memory response capable of preventing relapse.

In embodiments, the chimeric protein is capable of causing activation of antigen presenting cells. In embodiments, the chimeric protein is capable enhancing the ability of antigen presenting cells to present
15 antigen.

In embodiments, the present chimeric proteins are capable of, and can be used in methods comprising, transiently stimulating effector T cells for longer than about 12 hours, about 24 hours, about 48 hours, about 72 hours or about 96 hours or about 1 week or about 2 weeks. In embodiments, the transient stimulation of effector T cells occurs substantially in a patient's bloodstream or in a particular tissue/location including
20 lymphoid tissues such as for example, the bone marrow, lymph-node, spleen, thymus, mucosa-associated lymphoid tissue (MALT), non-lymphoid tissues, or in the tumor microenvironment.

In a chimeric protein of the present disclosure, the present chimeric protein unexpectedly provides binding of the extracellular domain components to their respective binding partners with slow off rates (K_d or K_{off}). In embodiments, this provides an unexpectedly long interaction of the receptor to ligand and vice versa. Such
25 an effect allows for a longer positive signal effect, *e.g.*, increase in or activation of immune stimulatory signals. For example, the present chimeric protein, *e.g.*, via the long off rate binding allows sufficient signal transmission to provide immune cell proliferation, allow for anti-tumor attack, allows sufficient signal transmission to provide release of stimulatory signals, *e.g.*, cytokines.

In a chimeric protein of the present disclosure, the chimeric protein is capable of forming a stable synapse
30 between cells. The stable synapse of cells promoted by the chimeric proteins (*e.g.*, between cells bearing negative signals) provides spatial orientation to favor tumor reduction - such as positioning the T cells to attack tumor cells and/or sterically preventing the tumor cell from delivering negative signals, including negative signals beyond those masked by the chimeric protein of the invention. In embodiments, this provides longer on-target (*e.g.*, intratumoral) half-life ($t_{1/2}$) as compared to serum $t_{1/2}$ of the chimeric proteins. Such

5 properties could have the combined advantage of reducing off-target toxicities associated with systemic distribution of the chimeric proteins.

In embodiments, the chimeric protein is capable of providing a sustained immunomodulatory effect.

The present chimeric proteins provide synergistic therapeutic effects (e.g., anti-tumor effects) as it allows for improved site-specific interplay of two immunotherapy agents. In embodiments, the present chimeric proteins
10 provide the potential for reducing off-site and/or systemic toxicity.

In embodiments, the present chimeric protein exhibit enhanced safety profiles. In embodiments, the present chimeric protein exhibit reduced toxicity profiles. For example, administration of the present chimeric proteins may result in reduced side effects such as one or more of diarrhea, inflammation (e.g., of the gut), or weight loss, which occur following administration of antibodies directed to the ligand(s)/receptor(s) targeted by the
15 extracellular domains of the present chimeric proteins. In embodiments, the present chimeric protein provides improved safety, as compared to antibodies directed to the ligand(s)/receptor(s) targeted by the extracellular domains of the present chimeric proteins, yet, without sacrificing efficacy.

In embodiments, the present chimeric proteins provide reduced side-effects, e.g., GI complications, relative to current immunotherapies, e.g., antibodies directed to ligand(s)/receptor(s) targeted by the extracellular
20 domains of the present chimeric proteins. Illustrative GI complications include abdominal pain, appetite loss, autoimmune effects, constipation, cramping, dehydration, diarrhea, eating problems, fatigue, flatulence, fluid in the abdomen or ascites, gastrointestinal (GI) dysbiosis, GI mucositis, inflammatory bowel disease, irritable bowel syndrome (IBS-D and IBS-C), nausea, pain, stool or urine changes, ulcerative colitis, vomiting, weight gain from retaining fluid, and/or weakness.

25 *Pharmaceutical composition*

Aspects of the present disclosure include a pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein as disclosed herein.

Any chimeric protein disclosed herein may be used in a pharmaceutical composition.

In embodiments, a chimeric protein disclosed herein is provided as a sterile frozen solution in a vial or as a
30 sterile liquid solution in a vial. A drug product comprising a chimeric protein disclosed herein comprises a sterile-filtered, formulated chimeric protein disclosed herein solution filled into a 10 mL single use glass vial stoppered with a Flurotec® rubber stopper and sealed with an aluminum flip off seal. In embodiments, a chimeric protein disclosed herein is formulated at between about 10mg/mL to about 30 mg/mL, e.g., about

5 20 mg/mL in between about 30 mM to about 70 mM L-histidine, e.g., about 50 mM L-histidine and between about 125 mM and about 400 mM sucrose, e.g., about 250 mM sucrose in water for injection. In embodiments, each vial contains about 1 mL of drug product or about 20 mg of a chimeric protein disclosed herein.

The chimeric proteins disclosed herein, including the CD172a (SIRP α)-Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61), can possess a sufficiently basic functional group, which can react with an inorganic or organic acid, or a carboxyl group, which can react with an inorganic or organic base, to form a pharmaceutically acceptable salt. A pharmaceutically-acceptable acid addition salt is formed from a pharmaceutically acceptable acid, as is well known in the art. Such salts include the pharmaceutically acceptable salts listed in, for example, *Journal of Pharmaceutical Science*, 66, 2-19 (1977) and *The Handbook of Pharmaceutical Salts; Properties, Selection, and Use*. P. H. Stahl and C. G. Wermuth (eds.), 15 Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety.

In embodiments, the compositions disclosed herein are in the form of a pharmaceutically acceptable salt.

Further, any chimeric protein disclosed herein can be administered to a subject as a component of a composition, e.g., pharmaceutical composition, that comprises a pharmaceutically acceptable carrier or vehicle. Such pharmaceutical compositions can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration. Pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical excipients can be, for example, saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, 20 auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used. In embodiments, the pharmaceutically acceptable excipients are sterile when administered to a subject. Water is a useful excipient when any agent disclosed herein is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, specifically for injectable solutions. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, 30 glycol, water, ethanol and the like. Any agent disclosed herein, if desired, can also comprise minor amounts of wetting or emulsifying agents, or pH buffering agents.

In embodiments, the compositions, e.g., pharmaceutical compositions, disclosed herein are resuspended in a saline buffer (including, without limitation TBS, PBS, and the like).

5 In embodiments, the chimeric proteins may be conjugated and/or fused with another agent to extend half-life or otherwise improve pharmacodynamic and pharmacokinetic properties. In embodiments, the chimeric proteins may be fused or conjugated with one or more of PEG, XTEN (e.g., as rPEG), polysialic acid (POLYXEN), albumin (e.g., human serum albumin or HAS), elastin-like protein (ELP), PAS, HAP, GLK, CTP, transferrin, and the like. In embodiments, each of the individual chimeric proteins is fused to one or more of
10 the agents described in Strohl, *BioDrugs* 29(4):215–239 (2015), the entire contents of which are hereby incorporated by reference.

The present disclosure includes the disclosed chimeric protein in various formulations of pharmaceutical composition. Any chimeric protein disclosed herein can take the form of solutions, suspensions, emulsion, drops, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations,
15 suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. DNA or RNA constructs encoding the protein sequences may also be used. In embodiments, the composition is in the form of a capsule (see, e.g., U.S. Patent No. 5,698,155). Other examples of suitable pharmaceutical excipients are described in *Remington's Pharmaceutical Sciences* 1447-1676 (Alfonso R. Gennaro eds., 19th ed. 1995), incorporated herein by reference.

20 Where necessary, the pharmaceutical compositions comprising the chimeric protein (can also include a solubilizing agent. Also, the agents can be delivered with a suitable vehicle or delivery device as known in the art. Compositions for administration can optionally include a local anesthetic such as, for example, lignocaine to lessen pain at the site of the injection.

The pharmaceutical compositions comprising the chimeric protein of the present disclosure may conveniently
25 be presented in unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods generally include the step of bringing therapeutic agents into association with a carrier, which constitutes one or more accessory ingredients. Typically, the pharmaceutical compositions are prepared by uniformly and intimately bringing therapeutic agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into dosage forms of the desired
30 formulation (e.g., wet or dry granulation, powder blends, etc., followed by tableting using conventional methods known in the art)

In embodiments, any chimeric protein disclosed herein is formulated in accordance with routine procedures as a pharmaceutical composition adapted for a mode of administration disclosed herein.

5 *Administration, Dosing, and Treatment Regimens*

An aspect of the present disclosure is a method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer. In embodiments, the method comprises: (i) obtaining a biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level and/or activity of a cell has been measured in a biological sample obtained from the subject before the administration of the first dose. In embodiments, the cell is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell. In embodiments, the method further comprises (ii) determining a post-dose level and/or activity of the cell in the biological sample. In embodiments, the method further comprises (iii) determining that the chimeric protein is efficacious if the post-dose level and/or activity of the cell is greater than the background level and/or activity of the cell. In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the level and/or activity of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

An aspect of the present disclosure is a method of selecting a subject for treatment with a therapy for a cancer. In embodiments, the method comprises: (i) obtaining a biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, a background level and/or activity of a cell has been measured in a biological sample obtained from the subject before the administration of the first dose. In embodiments, the cell is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell. In embodiments, the method further comprises (ii) determining a post-dose level and/or activity of the cell in the biological sample. In embodiments, the method further comprises (iii) selecting the subject for treatment with the chimeric protein if the post-dose level and/or activity of the cell is greater than the background level and/or activity of the cell.

5 In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the level and/or activity of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

In embodiments, the biological sample is selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating
10 nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the biological sample is a biopsy sample or a surgical specimen. In embodiments, the biological sample is a tumor biopsy sample or a tumor surgical specimen. In embodiments, the tumor biopsy sample or the tumor surgical specimen
15 derived from a tumor selected ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN).

In embodiments, the level and/or activity of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell
20 sorting (FACS) or a combination thereof. In embodiments, the level and/or activity of the cell is measured by contacting the sample with an agent that specifically binds to one or more molecules selected from CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof. In embodiments, the level and/or activity
25 of the cell is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding one or more of CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

An aspect of the present disclosure is a method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer. In embodiments, the method comprises: (i)
30 obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40

5 ligand (CD40L). In embodiments, the method further comprises determining a background level and/or activity of B cells and/or CD40+ cells in the first biological sample . In embodiments, the method further comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose level and/or activity of B cells and/or CD40+ cells in the second biological sample . In embodiments, 10 the method further comprises (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises determining an M-day post-dose level and/or activity of B cells and/or CD40+ cells in the third biological sample . In embodiments, the method further comprises (v) determining that the chimeric protein is efficacious if the N hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background 15 level and/or activity of B cells and/or CD40+ cells, and/or if the M day post-dose level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells.

An aspect of the present disclosure is a method of selecting a subject for treatment with a therapy for a cancer. In embodiments, the method comprises: (i) obtaining a first biological sample obtained from the 20 subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the method further comprises 25 determining a background level and/or activity of B cells and/or CD40+ cells in the first biological sample . In embodiments, the method further comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose level and/or activity of B cells and/or CD40+ cells in the second biological sample . In embodiments, the method further comprises (iv) obtaining a third biological sample 30 obtained from the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises determining an M-day post-dose level and/or activity of B cells and/or CD40+ cells in the third biological sample . In embodiments, the method further comprises (v) selecting the subject for treatment with the chimeric protein if the N hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells, and/or if the M day post-dose

5 level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells.

In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1. In embodiments, M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2. In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.

In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is greater than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells. In embodiments, the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is within about 10%, or about 20%, or about 30%, or about 40% of the background level and/or activity of B cells and/or CD40+ cells.

In embodiments, the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood.

25 In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof. In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to a CD40 and/or a B-cell marker. In embodiments, the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof. In embodiments, the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding a CD40 and/or a B-cell marker. In embodiments,

5 the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

An aspect of the present disclosure is a method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer. In embodiments, the method comprises: (i) obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the method further comprises (ii) determining in the first biological sample a background amount and/or activity of a cytokine. In embodiments, the cytokine is selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC. In embodiments, the method further comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose amount and/or activity of the cytokine in the second biological sample. In embodiments, the method further comprises (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises determining an M-day post-dose amount and/or activity of the cytokine in the third biological sample. In embodiments, the method further comprises (v) determining that the chimeric protein is efficacious if the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine, and/or if the M-day post-dose amount and/or activity of the cytokine is at least about 50% lower than the N-hour post-dose amount and/or activity of the cytokine.

An aspect of the present disclosure is a method of selecting a subject for treatment with a therapy for a cancer. In embodiments, the method comprises: (i) obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein. In embodiments, the chimeric protein has a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the method further comprises (ii) determining in the first biological sample a background amount and/or activity of a cytokine. In embodiments,

5 the cytokine is selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC. In embodiments, the method further comprises (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24. In embodiments, the method further comprises determining an N-hr post-dose amount and/or activity of the cytokine in the second biological sample. In embodiments, the method further comprises (iv) obtaining a third biological sample obtained from
 10 the subject at M days post-dose, wherein M is a number between 1 and 28. In embodiments, the method further comprises determining an M-day post-dose amount and/or activity of the cytokine in the third biological sample. In embodiments, the method further comprises (v) selecting the subject for treatment with the chimeric protein if the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine, and/or if the M-day post-dose amount and/or activity of the cytokine is
 15 at least about 50% lower than the N-hour post-dose amount and/or activity of the cytokine.

In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1. In embodiments, M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2. In embodiments, N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and M is less than 21, or less than 14, or
 20 less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.

In embodiments, the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine. In embodiments, the N-hr post-dose amount and/or activity of the cytokine is less than the background amount and/or activity of the cytokine. In embodiments, the N-hr post-dose amount and/or activity of the cytokine is within about 10%, or about 20%, or about 30%, or about 40%
 25 of the background amount and/or activity of the cytokine. In embodiments, the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or
 30 lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen. In embodiments, the first biological sample, the second biological sample and the third biological sample are blood.

In embodiments, the amount and/or activity of the cytokine is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and

5 fluorescent activating cell sorting (FACS) or a combination thereof. In embodiments, the amount and/or activity of the cytokine is measured by contacting the sample with an agent that specifically binds to the cytokine. In embodiments, the agent that specifically binds to the one or more molecules is an antibody or fragment thereof. In embodiments, the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof. In embodiments, the amount and/or activity of the cytokine is
10 measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding the cytokine. In embodiments, the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

In embodiments, the first dose of the chimeric protein is in the range of from about 0.03 mg/kg to 10 mg/kg. In embodiments, the first dose is about 0.003, or about 0.01, or about 0.03, or about 0.1, or about 0.3, or
15 about 1, or about 2, or about 3, or about 4, or about 6, or about 8, or about 10 mg/kg.

In embodiments, the method of any of the aspects disclosed herein further comprises administration of a second dose of the chimeric protein. In embodiments, the second dose is administered at least about 3 days, or at least about 4 days, or at least about 5 days, or at least about 6 days, or at least about 7 days, or at least
20 about 8 days, or at least about 9 days, or at least about 10 days, or at least about 14 days, or at least about 21 days, or at least about 28 days after the administration of the first dose. In embodiments, the second dose of the chimeric protein is in the range of from about 0.03 mg/kg to 10 mg/kg. In embodiments, the second dose is about 0.003, or about 0.01, or about 0.03, or about 0.1, or about 0.3, or about 1, or about 2, or about 3, or about 4, or about 6, or about 8, or about 10 mg/kg.

In embodiments, the method of any of the aspects disclosed herein further comprises administration of a
25 second dose of the chimeric protein. In embodiments, the second dose is administered at least about 3 days, or at least about 4 days, or at least about 5 days, or at least about 6 days, or at least about 7 days, or at least about 8 days, or at least about 9 days, or at least about 10 days, or at least about 14 days, or at least about 21 days, or at least about 28 days after the administration of the first dose. In embodiments, the second dose of the chimeric protein is in the range of from about 0.03 mg/kg to 10 mg/kg. In embodiments, the second
30 dose is about 0.003, or about 0.01, or about 0.03, or about 0.1, or about 0.3, or about 1, or about 2, or about 3, or about 4, or about 6, or about 8, or about 10 mg/kg.

In embodiments, the first domain is capable of binding a CD172a (SIRP α) ligand. In embodiments, the first domain comprises substantially all of the extracellular domain of CD172a (SIRP α). In embodiments, the first domain comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about

5 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 57.

In embodiments, the second domain is capable of binding a CD40 receptor. In embodiments, the second domain comprises substantially all of the extracellular domain of CD40L. In embodiments, the second domain comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%,
10 or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 58.

In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%,
15 or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, (a) the first domain comprises the amino acid sequence of SEQ ID NO: 57, (b) the second domain comprises the amino acid sequence of SEQ ID NO: 58, and (c) the linker comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or
20 or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7. In embodiments, the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 and SEQ ID NO: 7.

In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 90%, or at
25 least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.2% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO:
30 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.4% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.6% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.8% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO:

5 61. In embodiments, the chimeric protein comprises the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61.

In embodiments, the human subject suffers from or is suspected to suffer from an advanced solid tumor or a lymphoma. In embodiments, the human subject suffers from or is suspected to suffer from a cancer is selected from ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN). In embodiments, the human subject
10 has failed one or more platinum-based therapies. In embodiments, the human subject is ineligible for a platinum therapy. In embodiments, the human subject is not receiving a concurrent chemotherapy, immunotherapy, biologic or hormonal therapy, and/or wherein the human subject has received, been tolerant to, or is ineligible for standard therapy and/or the cancer has no approved therapy considered to be standard
15 of care.

In embodiments, a chimeric protein disclosed herein is presented as a sterile frozen solution at a concentration of about 20 mg/mL and a total volume of about 1 mL, optionally in a 10 mL glass vial. In embodiments, a chimeric protein disclosed herein is administered by intravenous (IV) infusion following dilution with normal saline. Starting dose, dose escalation schema and dose schedules of certain
20 embodiments are presented below.

In embodiments, the dose of the chimeric protein administered is at least 0.0001 mg/kg, e.g., between about 0.0001mg/kg and about 10 mg/kg. In embodiments, the dose of the chimeric protein administered is at least about 0.3 mg/kg, e.g., at least about 0.3 mg/kg, or about 1.0 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg, or about 8 mg/kg, or about 10 mg/kg. In embodiments, the dose of the chimeric
25 protein administered is at least about 1 mg/kg, e.g., at least about 1.0 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg, or about 8 mg/kg, or about 10 mg/kg. In embodiments, the doses of the SIRP α -Fc-CD40L chimeric protein are not limited by anemia or another cytopenia effects and are therefore higher than doses are allowed compared to certain other therapeutics (e.g. anti-CD47 antibodies or SIRP α Fc fusion protein). Further, in embodiments, a low dose priming is not needed.

30 In embodiments, the administration is intravenous. In embodiments, the administration is intratumoral. In embodiments, the administration is by injection. In embodiments, the administration is by infusion. In embodiments, the administration is performed by an intravenous infusion. In embodiments, the administration is performed by an intratumoral injection.

5 In embodiments, about the chimeric protein is administered at an initial dose (*e.g.*, *about* one of about 0.0001, about 0.001, about 0.003, about 0.01, about 0.03, about 0.1, about 0.3, about 1, about 2, about 3, about 4, about 6, about 8 or about 10 mg/kg) and the chimeric protein is administered in one or more subsequent administrations. In embodiments, about the one or more subsequent administrations has a dose of one or more of about 0.0001, about 0.001, about 0.003, about 0.01, about 0.03, about 0.1, about 0.3, about 1, about
10 2, about 3, about 4, about 6, about 8, about and about 10 mg/kg.

In embodiments, the starting dose and/or the subsequent doses is the maximum tolerated dose or less than the maximum tolerated dose.

In embodiments, the dose escalates between one or more subsequent dose in log increments, *e.g.*, 0.0001 mg/kg to 0.001 mg/kg, 0.001 mg/kg to 0.01 mg/kg, and 0.01 mg/kg to 0.1 mg/kg.

15 In embodiments, the dose escalates between one or more subsequent dose in about half log increments, *e.g.*, 0.001 mg/kg to 0.003 mg/kg and 0.003 mg/kg to 0.01 mg/kg.

In embodiments, the human subject has failed one or more platinum-based therapies, and optionally is ineligible for a platinum therapy. In embodiments, the human subject is not receiving a concurrent chemotherapy, immunotherapy, biologic or hormonal therapy, and/or wherein the human subject has
20 received, been tolerant to, or is ineligible for standard therapy and/or the cancer has no approved therapy considered to be standard of care.

In embodiments, the initial dose is less than the dose for at least one of the subsequent administrations., *e.g.*, each of the subsequent administrations.

In embodiments, the initial dose is the same as the dose for at least one of the subsequent administrations,
25 *e.g.*, each of the subsequent administrations.

In embodiments, the chimeric protein is administered at least about one time a month.

In embodiments, the chimeric protein is administered at least about two times a month.

In embodiments, the chimeric protein is administered at least about three times a month.

In embodiments, the chimeric protein is first administered once a week for three weeks and the chimeric
30 protein is then administered about once every three weeks or once every four weeks.

5 In embodiments, the chimeric protein is first administered once a week for three weeks and the chimeric protein is then administered about two times per month. For example, the chimeric protein is first administered once a week for three weeks and the chimeric protein is then administered about once every two weeks.

In embodiments, the chimeric protein is administered at least about four times a month. For example, the chimeric protein is administered about once a week. In embodiments, the chimeric protein is administered
10 once every week (once every seven days). In embodiments, the chimeric protein is administered once every two weeks.

In embodiments, the administration of the SIRP α -Fc-CD40L chimeric protein does not cause an anemia or another cytopenia in the patient. In embodiments, the administration of the does not cause lysis of RBCs. In embodiments, the administration of the SIRP α -Fc-CD40L chimeric protein is less likely to cause anemia or
15 another cytopenia in than, e.g. an anti-CD47 Ab. In embodiments, the doses of the SIRP α -Fc-CD40L chimeric protein are not limited by anemia or another cytopenia effects and are therefore higher than doses are allowed compared to certain other therapeutics (e.g. anti-CD47 antibodies or SIRP α Fc fusion protein). Further, in embodiments, a low dose priming is not needed.

Another advantage the SIRP α -Fc-CD40L chimeric protein (e.g. SEQ ID NO: 59 or SEQ ID NO: 61) offers is
20 that despite targeting does not cause an anemia or another cytopenia in the patient. This is because although the CD47/SIRP α interaction plays a key role in the lysis of RBCs, as shown herein, the SIRP α -Fc-CD40L chimeric protein does not cause lysis of RBCs. Accordingly, the present methods are less likely to cause anemia or another cytopenia in than, e.g. an anti-CD47 Ab.

A chimeric protein may be administered intravenously by intravenous infusion or bolus injection into the
25 bloodstream. A chimeric protein may be administered intravenously by intravenous infusion for patients suffering from advanced ovarian, fallopian tube and primary peritoneal cancers.

A chimeric protein may be administered an intratumoral injection. In embodiments, the therapeutic dose for intra-tumoral administration is equal or less than that of intravenous infusion. In embodiments, the therapeutic dose for intra-tumoral administration is equal to that of intravenous infusion. In embodiments, the therapeutic
30 dose for intra-tumoral administration is less than that of intravenous infusion. In embodiments, the therapeutic dose for intra-tumoral administration for patients suffering from advanced or metastatic CSCC and HNSCC.

In embodiments, the present chimeric protein allows for a dual effect that provides less side effects than are seen in conventional immunotherapy (e.g., treatments with one or more of OPDIVO, KEYTRUDA, YERVOY,

5 and TECENTRIQ). For example, the present chimeric proteins reduce or prevent commonly observed immune-related adverse events that affect various tissues and organs including the skin, the gastrointestinal tract, the kidneys, peripheral and central nervous system, liver, lymph nodes, eyes, pancreas, and the endocrine system; such as hypophysitis, colitis, hepatitis, pneumonitis, rash, and rheumatic disease.

10 Dosage forms suitable for intravenous administration include, for example, solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions (e.g., lyophilized composition), which can be dissolved or suspended in sterile injectable medium immediately before use. They may contain, for example, suspending or dispersing agents known in the art.

15 The dosage of any chimeric protein disclosed herein as well as the dosing schedule can depend on various parameters, including, but not limited to, the disease being treated, the subject's general health, and the administering physician's discretion.

In one aspect, the present disclosure relates to a method for treating a cancer in a human subject in need thereof, the method comprising a step of administering to the human subject an effective amount of a chimeric protein having a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L), wherein the step of administering comprises biphasic dosing. In embodiments, the first phase, and the second phase each independently comprise a dosing frequency of from about twice a week to about once every two months. In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond. In 25 embodiments, the chimeric protein exhibits a linear dose response in the dose range of e.g., about 0.3 mg/kg to about 3 mg/kg, or about 0.5 mg/kg to about 3 mg/kg, or about 0.7 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 3 mg/kg, or about 1.5 mg/kg to about 3 mg/kg, or about 2 mg/kg to about 3 mg/kg, or about 2.5 mg/kg to about 3 mg/kg, or about 0.3 mg/kg to about 2.5 mg/kg, or about 0.3 mg/kg to about 2 mg/kg, or about 0.3 mg/kg to about 1.5 mg/kg, or about 0.3 mg/kg to about 1.0 mg/kg, or about 0.3 mg/kg to about 0.5 30 mg/kg. In embodiments, the chimeric protein does not exhibit a bell-shaped dose response.

In embodiments, the dosing frequency of the first phase, and the dosing frequency of the second phase are the same. In other embodiments, the dosing frequency of the first phase, and the dosing frequency of the second phase are different.

5 In embodiments, the dosing frequency of the first phase is selected from about every three days, about twice a week, about every week, about every 10 days, about twice every 3 weeks, about every 2 weeks, about every 3 weeks, about every 4 weeks, about every month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the dosing frequency of the first phase is selected from about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, about 10 every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks, about every 5 weeks to about every 7 weeks, about every 6 weeks to about every 8 weeks, and , about every 6 weeks to about every 2 months.

In embodiments, the dosing frequency of the second phase is selected from about every three days, about 15 twice a week, about every week, about every 10 days, about twice every 3 weeks, about every 2 weeks, about every 3 weeks, about every 4 weeks, about every month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the dosing frequency of the second phase is selected from about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, 20 about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks, about every 5 weeks to about every 7 weeks, about every 6 weeks to about every 8 weeks, and , about every 6 weeks to about every 2 months.

In embodiments, the dosing frequency of the first phase is selected from from about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks; and 25 the frequency of the second phase is selected from from about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks.

Additionally, or alternatively, in embodiments, the first phase, and the second phase each independently last from about two days to about 12 months. In embodiments, the first phase lasts from about two weeks to 30 about 2 months; and the second phase lasts from about 2 weeks to about 12 months. In embodiments, the first phase lasts from about two weeks to about 1 month; and the second phase lasts from about 2 weeks to about 12 months. In embodiments, the first phase lasts from about two weeks to about 1 month; and the second phase lasts from about 4 weeks to about 12 months.

5 Additionally, or alternatively, in embodiments, the effective amount for the first phase, the second phase and the third phase each independently comprise about 0.01 mg/kg to about 10 mg/ml. In embodiments, the effective amount for the first phase, the second phase and the third phase each independently selected from about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 3 mg/kg, about 10 mg/kg, and any range including and/or in between any two of the preceding values. In embodiments, the effective amount
10 for the first phase, the second phase and the third phase each independently selected from from about 0.01 mg/kg to about 0.1 mg/kg, about 0.03 mg/kg to about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.3 mg/kg to about 3 mg/kg, and about 1 mg/kg to about 10 mg/kg. In embodiments, the effective amount for the first phase, the second phase and the third phase are same. In embodiments, the effective amount for the first phase, the second phase and the third phase are different. In embodiments, the effective amount for
15 the first phase is greater than the effective amount for the second phase. In embodiments, the effective amount for the first phase is from about 0.03 mg/kg to about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.3 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 10 mg/kg; and the effective amount for the second phase is from about 0.01 mg/kg to about 0.1 mg/kg, about 0.03 mg/kg to about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.3 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 10 mg/kg.

20 In embodiments, the chimeric proteins disclosed herein is the human CD172a (SIRP α)-Fc-CD40L chimeric protein.

In one aspect, the present disclosure relates to a method for treating a cancer in a human subject in need thereof the method comprising a step of administering to the human subject an effective amount of a chimeric protein having a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain
25 comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L), wherein the step of administration comprises a first cycle, a second cycle and a third cycle. In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond. In embodiments, the first cycle, the second
30 cycle and the third cycle each independently comprise a dosing frequency of from about twice a week to about once every two months. In embodiments, the dosing frequency of the first cycle, the dosing frequency of the second cycle and the dosing frequency of the third cycle are the same. In embodiments, the dosing frequency of the first cycle, the dosing frequency of the second cycle and the dosing frequency of the third cycle are different. In embodiments, the dosing frequency of the first cycle is selected from about every three
35 days, about twice a week, about every week, about every 10 days, about twice every 3 weeks, about every

5 2 weeks, about every 3 weeks, about every 4 weeks, about every month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the chimeric protein exhibits a linear dose response in the dose range of e.g., about 0.3 mg/kg to about 3 mg/kg, or about 0.5 mg/kg to about 3 mg/kg, or about 0.7 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 3 mg/kg, or about 1.5 mg/kg to about 3 mg/kg, or about 2 mg/kg to about 3 mg/kg, or about 2.5 mg/kg to about 3 mg/kg, or about 0.3 mg/kg to about 2.5 mg/kg, or about 0.3 mg/kg to about 2 mg/kg, or about 0.3 mg/kg to about 1.5 mg/kg, or about 0.3 mg/kg to about 1.0 mg/kg, or about 0.3 mg/kg to about 0.5 mg/kg. In
10 embodiments, the chimeric protein does not exhibit a bell-shaped dose response.

In embodiments, the dosing frequency of the first cycle is selected from about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every
15 2 weeks to about every 4 weeks, about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks, about every 5 weeks to about every 7 weeks, about every 6 weeks to about every 8 weeks, and , about every 6 weeks to about every 2 months. In embodiments, the dosing frequency of the second cycle is selected from about every three days, about twice a week, about every week, about every 10 days, about twice every 3 weeks, about every 2 weeks, about every 3 weeks, about every 4 weeks, about every
20 month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the dosing frequency of the second cycle is selected from about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks, about every 5 weeks to about every 7 weeks, about every 6 weeks
25 to about every 8 weeks, and , about every 6 weeks to about every 2 months. In embodiments, the dosing frequency of the third cycle is selected from about every three days, about twice a week, about every week, about every 10 days, about twice every 3 weeks, about every 2 weeks, about every 3 weeks, about every 4 weeks, about every month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the dosing frequency of the third cycle is selected from
30 about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks, about every 5 weeks to about every 7 weeks, about every 6 weeks to about every 8 weeks, and , about every 6 weeks to about every 2 months. In embodiments, the dosing frequency of the first cycle is selected from from about every 3 days to about every 10 days, about
35 every week to about every 2 weeks, about every 10 days to about every 3 weeks; and the frequency of the

5 second cycle is selected from from about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks.

Additionally, or alternatively, in embodiments, the first cycle, the second cycle and the third cycle each independently last from about two days to about 12 months. In embodiments, the first cycle lasts from about
10 two weeks to about 2 months; and the second cycle lasts from about 2 weeks to about 12 months. In embodiments, the first cycle lasts from about two weeks to about 2 months; the second cycle lasts from about 2 weeks to about 12 months and the third cycle lasts from about 2 weeks to about 6 months.

Additionally, or alternatively, in embodiments, the effective amount for the first cycle, the second cycle and the third cycle each independently comprise about 0.01 mg/kg to about 10 mg/ml. In embodiments, the
15 effective amount for the first cycle, the second cycle and the third cycle each independently selected from about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 3 mg/kg, about 10 mg/kg, and any range including and/or in between any two of the preceding values. In embodiments, the effective amount for the first cycle, the second cycle and the third cycle each independently selected from from about 0.01 mg/kg to about 0.1 mg/kg, about 0.03 mg/kg to about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about
20 0.3 mg/kg to about 3 mg/kg, and about 1 mg/kg to about 10 mg/kg.

In embodiments, the effective amount for the first cycle, the second cycle and the third cycle are same. In other embodiments, the effective amount for the first cycle, the second cycle and the third cycle are different. In embodiments, the effective amount for the first cycle is greater than the effective amount for the second cycle. In other embodiments, the effective amount for the first cycle is lesser than the effective amount for
25 the second cycle. In yet other embodiments, the effective amount for the first cycle and the effective amount for the second cycle are the same.

In embodiments, the effective amount for the first cycle is from about 0.03 mg/kg to about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.3 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 10 mg/kg; and the effective amount for the second cycle is from about 0.01 mg/kg to about 0.1 mg/kg, about 0.03 mg/kg to
30 about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.3 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 10 mg/kg.

In embodiments, the chimeric proteins disclosed herein is the human CD172a (SIRP α)-Fc-CD40L chimeric protein.

5 In one aspect, the present disclosure relates to a method for treating a cancer in a human subject in need thereof the method comprising a step of administering to the human subject an effective amount of an effective amount of a chimeric protein having a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40
10 ligand (CD40L) with a dosing regimen, wherein the dosing regimen comprises dosing with a frequency in the range of about every three days to about every 2 months. In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond. In embodiments, the dosing regimen is selected from about every three days, about twice a week, about every week, about every 10 days, about twice every 3
15 weeks, about every 2 weeks, about every 3 weeks, about every 4 weeks, about every month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the dosing regimen is selected from about every week, about every 10 days, about every 2 weeks, about every 3 weeks, about every 4 weeks, about every month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the dosing
20 regimen is about every 2 weeks, about every 3 weeks, or about every 4 weeks.

In one aspect, the present disclosure relates to a method for treating a cancer in a human subject in need thereof the method comprising a step of administering to the human subject an effective amount of an effective amount of a chimeric protein having a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40
25 ligand (CD40L) with a dosing regimen selected from about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks, about every 5 weeks to about every 7 weeks, about every 6 weeks to about every 8 weeks, and , about every
30 6 weeks to about every 2 months. In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond. In embodiments, the dosing regimen is about every week to about every 2 weeks, about every 10 days to about every 3 weeks, or about every 2 weeks to about every 4 weeks. In embodiments, the chimeric protein exhibits a linear dose response in the dose range of e.g., about 0.3 mg/kg
35 to about 3 mg/kg, or about 0.5 mg/kg to about 3 mg/kg, or about 0.7 mg/kg to about 3 mg/kg, or about 1

5 mg/kg to about 3 mg/kg, or about 1.5 mg/kg to about 3 mg/kg, or about 2 mg/kg to about 3 mg/kg, or about 2.5 mg/kg to about 3 mg/kg, or about 0.3 mg/kg to about 2.5 mg/kg, or about 0.3 mg/kg to about 2 mg/kg, or about 0.3 mg/kg to about 1.5 mg/kg, or about 0.3 mg/kg to about 1.0 mg/kg, or about 0.3 mg/kg to about 0.5 mg/kg. In embodiments, the chimeric protein does not exhibit a bell-shaped dose response.

In some embodiments of any of the aspects disclosed herein, the first domain is capable of binding a CD172a (SIRP α) ligand. In embodiments, the first domain comprises substantially all of the extracellular domain of CD172a (SIRP α). In embodiments, the second domain is capable of binding a CD40 receptor. In
10 In embodiments, the second domain comprises substantially all of the extracellular domain of CD40L. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, e.g., human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or
15 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or
20 SEQ ID NO: 3. In embodiments, the first domain comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain comprises an amino acid sequence that is at least 96% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO:
25 57. In embodiments, the first domain comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the first domain comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 57.

In some embodiments of any of the aspects disclosed herein, the second domain comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence
30 of SEQ ID NO: 58. In some embodiments of any of the aspects disclosed herein, the second domain comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments of any of the aspects disclosed herein, the second domain comprises an amino acid sequence that is at least 96% identical to the amino acid sequence of SEQ ID NO: 58. In some
35 embodiments of any of the aspects disclosed herein, the second domain comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments of any of

5 the aspects disclosed herein, the second domain comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments of any of the aspects disclosed herein, the second domain comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments of any of the aspects disclosed herein, the second domain comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 58.

10 In embodiments, (a) the first domain comprises the amino acid sequence of SEQ ID NO: 57, (b) the second domain comprises the amino acid sequence of SEQ ID NO: 58, and (c) the linker comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7. In embodiments, the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 and SEQ ID NO: 7. In embodiments, the chimeric protein comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least 96% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least 97% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least 99% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 98% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99% identical to SEQ ID NO: 59 or SEQ ID NO: 61.

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Additionally or alternatively, in embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.2% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.4% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.6% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is at least about 99.8% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61. In

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5 embodiments, the human subject has received, been tolerant to, or is ineligible for standard therapy and/or the cancer has no approved therapy considered to be standard of care.

In one aspect, the present disclosure relates to a method for promoting the migration of lymphocytes from peripheral blood into secondary lymphoid organs (e.g. the lymph nodes and spleen in a human subject in need thereof, the method comprising a step of administering to the human subject an effective amount of a
10 chimeric protein having a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L).

In some embodiments of any of the aspects disclosed herein, the human subject is not receiving a concurrent
15 chemotherapy, immunotherapy, biologic or hormonal therapy.

In one aspect, the present disclosure relates to a chimeric protein for use in the method of any of the embodiments disclosed herein.

In one aspect, the present disclosure relates to a chimeric protein comprising an amino acid sequence that is at least about 98% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein
20 comprises an amino acid sequence that is at least about 99% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the chimeric protein comprises an amino acid sequence that is identical to SEQ ID NO: 59 or SEQ ID NO: 61.

The dosing frequency of the first phase, and the dosing frequency of the second phase may be same or different. In embodiments, the dosing frequency of the first phase and the dosing frequency of the second
25 phase are each independently selected from about every three days, about twice a week, about every week, about every 10 days, about twice every 3 weeks, about every 2 weeks, about every 3 weeks, about every 4 weeks, about every month, about every 5 weeks, about every 6 weeks, about 7 seven weeks, about every 8 weeks and about every 2 months. In embodiments, the dosing frequency of the first phase is selected from
30 about every 3 days to about every 10 days, about every week to about every 2 weeks, about every 10 days to about every 3 weeks, about every 2 weeks to about every 4 weeks, about every 3 weeks to about every 5 weeks, about every 4 weeks to about every 6 weeks, about every 5 weeks to about every 7 weeks, about every 6 weeks to about every 8 weeks, and , about every 6 weeks to about every 2 months.

5 In embodiments, the first phase, and the second phase each independently last from about two days to about 12 months. For example, In embodiments, the first phase lasts from about two weeks to about 2 months; and the second phase lasts from about 2 weeks to about 12 months. In embodiments, the first phase lasts from about two weeks to about 1 month; and the second phase lasts from about 2 weeks to about 12 months. In
10 about 4 weeks to about 12 months.

The effective amount for the first phase, the second phase and the third phase may be same or different. In embodiments, the effective amount for the first phase, the second phase and the third phase each independently comprise about 0.01 mg/kg to about 10 mg/ml. In embodiments, the effective amount for the first phase is from about 0.03 mg/kg to about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.3 mg/kg
15 to about 3 mg/kg, or about 1 mg/kg to about 10 mg/kg; and the effective amount for the second phase is from about 0.01 mg/kg to about 0.1 mg/kg, about 0.03 mg/kg to about 0.3 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.3 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 10 mg/kg. In embodiments, the chimeric proteins disclosed herein is the human CD172a (SIRP α)-Fc-CD40L chimeric protein.

In embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein is capable of providing a sustained
20 immunomodulatory effect.

In embodiments, the linker comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino
25 acid sequence that is at least 96% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In embodiments, the linker comprises an amino acid sequence that is
30 identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the linker comprises hinge-CH2-CH3 Fc domain derived from IgG. In embodiments, the linker comprises hinge-CH2-CH3 Fc domain derived from an IgG selected from IgG1 and IgG4. In embodiments, the linker comprises hinge-CH2-CH3 Fc domain derived from human IgG1 or human IgG4. In

5 embodiments, the linker comprises hinge-CH2-CH3 Fc domain derived from IgG4. In embodiments, the hinge-CH2-CH3 Fc domain is derived from human IgG4.

Additionally, or alternatively, in embodiments, the extracellular domain of human signal regulatory protein α (CD172a (SIRP α)) comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the extracellular domain of
10 human signal regulatory protein α (CD172a (SIRP α)) comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the extracellular domain of human signal regulatory protein α (CD172a (SIRP α)) comprises an amino acid sequence that is at least 96% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the extracellular domain of human signal regulatory protein α (CD172a (SIRP α)) comprises an amino acid sequence that is at least 98%
15 identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the extracellular domain of human signal regulatory protein α (CD172a (SIRP α)) comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 57. In embodiments, the extracellular domain of human signal regulatory protein α (CD172a (SIRP α)) comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 57.

20 Additionally, or alternatively, in embodiments, the extracellular domain of human CD40 ligand (CD40L) comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the extracellular domain of human CD40 ligand (CD40L) comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the extracellular domain of human CD40 ligand (CD40L) comprises an
25 amino acid sequence that is at least 96% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the extracellular domain of human CD40 ligand (CD40L) comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the extracellular domain of human CD40 ligand (CD40L) comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 58. In embodiments, the extracellular domain of human CD40 ligand
30 (CD40L) comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 58.

Additionally, or alternatively, in embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein comprises an amino acid sequence that is at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 59 or SEQ ID NO:

5 61. In embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein comprises an amino acid sequence that is at least 96% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein comprises an amino acid sequence that is at least 97% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein comprises an amino acid sequence that is at least 99% identical to SEQ ID NO: 59 or SEQ ID NO: 61. In embodiments, the human CD172a (SIRP α)-Fc-CD40L chimeric protein comprises an amino acid sequence that is identical to SEQ ID NO: 59 or SEQ ID NO: 61.

In one aspect, the present disclosure relates to a method for treating a cancer in a human subject comprising:

15 (i) administering to the human subject a chimeric protein having a general structure of: N terminus – (a) – (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L); and (ii) administering a second therapeutic agent. In embodiments, the chimeric protein is administered at a dose between about 0.0001 mg/kg and about 10 mg/kg. In embodiments, the dose of the chimeric protein administered is at least about 0.3 mg/kg, e.g., at least about 0.3 mg/kg, or about 1.0 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg, or about 8 mg/kg, or about 10 mg/kg. In embodiments, the dose of the chimeric protein administered is at least about 1 mg/kg, e.g., at least about 1.0

20 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg, or about 8 mg/kg, or about 10 mg/kg. In embodiments, the chimeric protein exhibits a linear dose response in the dose range of e.g., about 0.3 mg/kg to about 3 mg/kg, or about 0.5 mg/kg to about 3 mg/kg, or about 0.7 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 3 mg/kg, or about 1.5 mg/kg to about 3 mg/kg, or about 2 mg/kg to about 3 mg/kg, or about 2.5 mg/kg to about 3 mg/kg, or about 0.3 mg/kg to about 2.5 mg/kg, or about 0.3 mg/kg to about 2 mg/kg, or about 0.3 mg/kg to about 1.5 mg/kg, or about 0.3 mg/kg to about 1.0 mg/kg, or about 0.3 mg/kg to about 0.5

25 mg/kg. In embodiments, the chimeric protein does not exhibit a bell-shaped dose response.

In embodiments, the administration of the chimeric protein causes a CD47 receptor occupancy (RO) on leukocytes that is at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about

30 85%, or at least about 90%, or at least about 95% compared to the RO prior to administration of the chimeric

5 protein, a second subject that is not administered the chimeric protein and/or an external control. In
embodiments, the administration of the chimeric protein causes a CD47 receptor occupancy (RO) on B cells
that is at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least
about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at
least about 90%, or at least about 95% compared to the RO prior to administration of the chimeric protein, a
10 second subject that is not administered the chimeric protein and/or an external control. In embodiments, the
administration of the chimeric protein causes an increase in the amount or activity of one or more of IL-12,
MCP-1, MIP-1 β , MIP-1 α , and MDC % compared to the RO prior to administration of the chimeric protein, a
second subject that is not administered the chimeric protein and/or an external control.

In one aspect, the present disclosure relates to a method for treating a cancer in a human subject comprising
15 administering to a subject in need thereof: 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 human signal
regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the
linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-
CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand
20 (CD40L); wherein: the subject is undergoing or has undergone treatment with a second therapeutic agent. In
embodiments, the chimeric protein is administered at a dose between about 0.0001 mg/kg and about 10
mg/kg. In embodiments, the dose of the chimeric protein administered is at least about 0.3 mg/kg, e.g., at
least about 0.3 mg/kg, or about 1.0 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg, or
about 8 mg/kg, or about 10 mg/kg. In embodiments, the dose of the chimeric protein administered is at least
25 about 1 mg/kg, e.g., at least about 1.0 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg,
or about 8 mg/kg, or about 10 mg/kg. In embodiments, the chimeric protein exhibits a linear dose response
in the dose range of e.g., about 0.3 mg/kg to about 3 mg/kg, or about 0.5 mg/kg to about 3 mg/kg, or about
0.7 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 3 mg/kg, or about 1.5 mg/kg to about 3 mg/kg, or
about 2 mg/kg to about 3 mg/kg, or about 2.5 mg/kg to about 3 mg/kg, or about 0.3 mg/kg to about 2.5 mg/kg,
30 or about 0.3 mg/kg to about 2 mg/kg, or about 0.3 mg/kg to about 1.5 mg/kg, or about 0.3 mg/kg to about 1.0
mg/kg, or about 0.3 mg/kg to about 0.5 mg/kg. In embodiments, the chimeric protein does not exhibit a bell-
shaped dose response.

In one aspect, the present disclosure relates to a method for treating a cancer in a human subject comprising
administering to a subject in need thereof a second anticancer therapeutic agent, wherein the subject is
35 undergoing or has undergone treatment with a chimeric protein of a general structure of N terminus – (a) –

5 (b) – (c) – C terminus, wherein: (a) is a first domain comprising an extracellular domain of human Signal regulatory protein α (CD172a (SIRP α)), (b) is a linker adjoining the first and second domains, wherein the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L). In embodiments, the chimeric protein is administered at a dose between about 0.0001 mg/kg and
 10 about 10 mg/kg. In embodiments, the chimeric protein exhibits a linear dose response in the dose range of e.g., about 0.3 mg/kg to about 3 mg/kg, or about 0.5 mg/kg to about 3 mg/kg, or about 0.7 mg/kg to about 3 mg/kg, or about 1 mg/kg to about 3 mg/kg, or about 1.5 mg/kg to about 3 mg/kg, or about 2 mg/kg to about 3 mg/kg, or about 2.5 mg/kg to about 3 mg/kg, or about 0.3 mg/kg to about 2.5 mg/kg, or about 0.3 mg/kg to about 2 mg/kg, or about 0.3 mg/kg to about 1.5 mg/kg, or about 0.3 mg/kg to about 1.0 mg/kg, or about 0.3
 15 mg/kg to about 0.5 mg/kg. In embodiments, the chimeric protein does not exhibit a bell-shaped dose response.

In embodiments, the chimeric protein is administered before the second therapeutic agent. In embodiments, the second therapeutic agent is administered before the chimeric protein. In embodiments, the second therapeutic agent and the chimeric protein are administered substantially together.

20 In embodiments, the second therapeutic agent is selected from an antibody, and a chemotherapeutic agent. In embodiments, the antibody is capable of antibody-dependent cellular cytotoxicity (ADCC). In embodiments, the antibody is selected from cetuximab, rituximab, obinutuzumab, Hu14.18K322A, Hu3F8, dinituximab, and trastuzumab. In embodiments, the antibody is capable of antibody-dependent cellular phagocytosis (ADCP). In embodiments, the antibody is selected from cetuximab, daratumumab, rituximab,
 25 and trastuzumab. In embodiments, the antibody is capable of binding a molecule selected from carcinoembryonic antigen (CEA), EGFR, HER-2, epithelial cell adhesion molecule (EpCAM), and human epithelial mucin-1, CD20, CD30, CD38, CD40, and CD52. In embodiments, the antibody is capable of binding EGFR. In embodiments, the antibody is selected from Mab A13, AMG595, cetuximab (Erbix, C225), panitumumab (ABX-EGF, Vectibix), depatuxizumab (ABT 806), depatuxizumab, mafodotin, duligotuzumab
 30 (MEHD7945A, RG7597), Futuximab (Sym004), GC1118, imgatuzumab (GA201), matuzumab (EMD 72000), necitumumab (Portrazza), nimotuzumab (h-R3), anitumumab (Vectibix, ABX-EGF), zalutumumab, humMR1, and tomuzotuximab. In embodiments, the antibody is cetuximab.

5 In embodiments, the chemotherapeutic agent is an anthracycline. In embodiments, the anthracycline is selected from doxorubicin, daunorubicin, epirubicin and idarubicin, and pharmaceutically acceptable salts, acids or derivatives thereof. In embodiments, the chemotherapeutic agent is doxorubicin.

In embodiments, the dose of the chimeric protein administered is at least about 0.0001 mg/kg, *e.g.*, between about 0.0001 mg/kg and about 10.0 mg/kg. The chimeric protein may be administered at an initial dose (*e.g.*,
10 at one of about 0.0001, about 0.001, about 0.003, about 0.01, about 0.03, about 0.1, about 0.3, about 1, about 2, about 3, about 4, about 6 or about 10.0 mg/kg) and the chimeric protein is administered in one or more subsequent administrations (*e.g.*, at one or more of about 0.0001, about 0.001, about 0.003, about 0.01, about 0.03, about 0.1, about 0.3, about 1, about 2, about 3, about 4, about 6, about 8, and about 10 mg/kg). In
15 embodiments, the dose of the chimeric protein administered is at least about 0.3 mg/kg, *e.g.*, at least about 0.3 mg/kg, or about 1.0 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg, or about 8 mg/kg, or about 10 mg/kg. In embodiments, the dose of the chimeric protein administered is at least about 1 mg/kg, *e.g.*, at least about 1.0 mg/kg, or about 2 mg/kg, or about 3, about 4 mg/kg, or about 6 mg/kg, or about 8 mg/kg, or about 10 mg/kg. In embodiments, the initial dose is less than the dose for at least one of the
20 subsequent administrations (*e.g.* each of the subsequent administrations) or the initial dose is the same as the dose for at least one of the subsequent administrations (*e.g.*, each of the subsequent administrations). In embodiments, the starting dose and/or the subsequent doses is the maximum tolerated dose or less than the maximum tolerated dose. In embodiments, the chimeric protein is administered at least about one time a month, *e.g.*, at least about two times a month, at least about three times a month, and at least about four times a month. In embodiments, the chimeric protein is first administered once a week for three weeks and
25 the chimeric protein is then administered about once every three weeks or once every four weeks; alternately, the chimeric protein is first administered once a week for three weeks and the chimeric protein is then administered about two times per month, *e.g.*, once a week for three weeks and the chimeric protein is then administered about once every two weeks.

In embodiments, the cancer comprises an advanced solid tumor (local and/or metastatic) or a lymphoma. In
30 embodiments, the cancer is selected from ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN). In embodiments, the cancer comprises an advanced solid tumor (local and/or metastatic) or advanced lymphoma.

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EXAMPLES

The examples herein are provided to illustrate advantages and benefits of the present technology and to further assist a person of ordinary skill in the art with preparing or using the chimeric proteins of the present technology. The examples herein are also presented in order to more fully illustrate the preferred aspects of the present technology. The examples should in no way be construed as limiting the scope of the present technology, as defined by the appended claims. The examples can include or incorporate any of the variations, aspects or embodiments of the present technology described above. The variations, aspects or embodiments described above may also further each include or incorporate the variations of any or all other variations, aspects or embodiments of the present technology.

Example 1: Phase 1 Clinical Trial of the SIRP α -Fc-CD40L Chimeric Protein (SL-172154)

This first-in-human Phase 1 dose escalation study is currently evaluating SL-172154 as monotherapy in subjects with platinum resistant ovarian cancer. Primary Objectives of the study are to evaluate safety; identify the maximum tolerated dose or maximum administered dose of SL-172154. Secondary Objectives of the study are to identify a dose and schedule (i.e., a recommended phase 2 dose [RP2D]), to characterize the PK and immunogenicity, and to evaluate anti-tumor activity per RECISTv1.1 for solid tumors. Exploratory Objectives are: to assess receptor occupancy of SIRP α and CD40 on PBMCs, and to investigate pharmacodynamic (PD) effects in blood and tumor.

The planned dose escalation is in half-log increments (**FIG. 1**). At least 3 subjects were enrolled into sequential dose levels (DL) and evaluated for dose limiting toxicity (DLT) in the first cycle of treatment. Subjects receive intravenous (IV) administration of SL-172154 on Schedule 1 or Schedule 2 until disease progression, unacceptable toxicity, or withdrawal of consent. Currently enrolling 10mg/kg.

Key Inclusion Criteria are:

- Locally advanced or metastatic ovarian cancer, primary peritoneal cancer or fallopian tube cancer.
- Refractory to existing therapy(ies) and ineligible for a platinum therapy. Subjects with homologous recombination deficiency positive disease must have received prior PARPi with or without bevacizumab.
- Age 18 years or older
- ECOG performance status of 0 or 1

- 5 • Measurable disease per RECIST v1.1

Key Exclusion Criteria are:

- Primary platinum refractory as defined by progressing during or within 1 month of upfront platinum therapy
- Prior treatment with an anti-CD47 or anti-SIRPα targeting agent or a CD40 agonist.
- 10 • Documented history of autoimmune disease or active pneumonitis
- Concurrent use of systemic corticosteroids or other immunosuppressive medication

Subject Characteristics are shown in **Table 4**:

Table 4. Tumor Characteristics

Total number of subjects, n (%)		N = 15
Cancer type	Ovarian cancer	9 (60)
	Primary peritoneal cancer	4 (27)
	Fallopian tube cancer	2 (13)
FIGO stage FIGO = International Federation of Gynecology and Obstetrics	Stage III	6 (40)
	Stage IV	9 (60)
Grade	High grade	11 (73)
	Grade 3	1 (7)
	Unknown	3 (20)
Histologic classification	Serous carcinoma	11 (73)
	Adenocarcinoma	2 (13)
	Serous adenocarcinoma	2 (13)

- 15 15 subjects of median age 67 years (range 33-79), having received a median of 5 prior lines of systemic therapy (range 2- 7) were treated with IV SL-172154 across 4 dose levels on 2 schedules. 13 of 15 subjects had an ECOG PS of 1 at baseline. 6 subjects were treated on schedule 1 (days 1, 8, 15, 29, q2wks) at 0.1 mg/kg (n=3) and 0.3 mg/kg (n=3). 9 subjects treated on schedule 2 (once weekly) at 0.3 mg/kg (n=3), 1.0 mg/kg (n=3), and 3.0 mg/kg (n=3). Most subjects had primary ovarian cancer (n=9; 60%), FIGO stage IV
- 20 (n=9; 60%), high grade disease (n=11; 73%) and serous carcinoma histology (n=11; 73%) as shown in **Table 1**. Adverse events are listed in **Table 5**.

Table 5. Most Common AEs in All Treated Subjects

Total number of subjects, n (%)	N = 15
Any AE	15 (100)
Fatigue	9 (60)
IRR	8 (53)
Nausea	5 (33)
Diarrhea	4 (27)
Decreased appetite	3 (20)
Dehydration	3 (20)
Pruritus	3 (20)
Abdominal distention	2 (13)
Back pain	2 (13)
Chills	2 (13)
Constipation	2 (13)
Dyspnea	2 (13)
Hypomagnesemia	2 (13)
Injection site reaction	2 (13)
Vomiting	2 (13)

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No DLTs have been observed with SL-172154 on either schedule at doses ranging from 0.1 mg/kg to 3.0 mg/kg. 15 subjects (100%) experienced an adverse event (AE) on treatment. The most common AEs, occurring in ≥ 3 subjects (all causality) were fatigue, infusion-related reaction (IRR), nausea, diarrhea, decreased appetite, dehydration, and pruritus (**Table 2**). 14 subjects (93%) had treatment-related AEs (TRAES). The most common TRAES, occurring in ≥ 2 subjects, were IRR (n=8; 53%) fatigue (n=7; 47%), nausea (n=4; 27%), decreased appetite (n=3; 20%), chills (n=2; 13%), diarrhea (n=2; 13%) and dyspnea (n=2, 13%). No $\geq$ G3 TRAES have been reported. 8 subjects (53%) had infusion-related reactions (IRRs) dosed at 0.3 mg/kg (n=3), 1.0 mg/kg (n=2) and 3.0 mg/kg (n=3); 1 IRR event was G3 and deemed secondary to iron infusion, 14 IRRs were G2 in severity and 2 IRRs were G1 in severity. IRRs were manageable with pre-medications, did not prevent completion of IV dosing or lead to discontinuation of SL- 172154. 13 subjects (87%) subjects have discontinued SL-172154; 12 discontinued due to radiological or clinical progression and 1 subject elected to stop treatment.

15

FIG. 3 shows the tumor response and duration of treatment. Best response among 14 efficacy evaluable subjects with post-baseline scan were:

20

- SD, n=4
- PD, n=9
- NE, n=1 (Subject had SD but did not meet protocol-specified minimum interval for SD)
- 1 subject (3 mg/kg) had not reached the first on-treatment disease assessment at week 8

5 Pharmacokinetics:

- Maximum concentration (C_{max}) and Area under the curve, time 0 extrapolated to infinity (AUC_{inf}) increased disproportionately with increasing dose.
- Clearance decreased with increasing dose.
- PK appeared to be biphasic at 1.0 and 3.0 mg/kg.
- 10 • Elimination phase of the curve was not fully characterized at the doses studied.

FIG. 4A to FIG. 4D show the reproducible increases in serum cytokines following repeated dosing of SL-172154. Plasma was prepared from subject blood draws at the indicated times. Cytokine levels were assayed using a multiplexed ECL ELISA method and select dose level cytokines are shown. Proinflammatory chemokines, CCL2 (MCP-1) (**FIG. 4A**), CCL4 (MIP-1 β) (**FIG. 4B**), CCL3 (MIP-1 α) (**FIG. 4C**), and CCL22 (MDC) (**FIG. 4D**) exhibited dose-dependent increases in plasma levels at all post infusion measurements. These increases were of equal duration and magnitude across all infusion intervals.

FIG. 5A and FIG. 5B show the dose-dependent and reproducible increases in Serum IL-12. **FIG. 5A** shows the subject level interleukin 12 (IL-12), a mediator of TH1 proinflammatory responses over time typify the cyclic effector cytokine responses observed in study subjects. **FIG. 5B** shows median responses at the first infusion (horizontal bars), preliminarily appear to be dose dependent.

FIG. 6A and FIG. 6B demonstrate the SL-172154 preferentially binds CD47 on leukocytes but not RBCs. **FIG. 6A** shows the CD47 receptor occupancy (RO) as evaluated by fluorescence activated cell sorting (FACS) analysis using whole blood on both red blood cells (RBC) and white blood cells (WBC, leukocytes). At one-hour post infusion on Cycle 1 Day 1 (C1D1), median CD47 RO on leukocytes (horizontal bars) is ~80%. **FIG. 6B** shows the CD47 RO on RBC is <5% for all dose levels.

FIG. 7A to FIG. 7C demonstrate that SL-172154 stimulates dose-dependent B cell margination and activation. B cells represent a large pool of circulating immune cells expressing high levels of CD40. Fluorescence activated cell sorting (FACS) panels were designed to interrogate CD40 receptor occupancy and states of activation and maturation. On C1D1, nearly all (~80%) CD40+ B cells marginate, or exit the circulation, within one-hour post-infusion. **FIG. 7A** shows the median frequency of marginating cells increases in a dose-dependent manner (horizontal bars). Receptor engagement is ~100% at all dose levels (data not shown). **FIG. 7B** shows the median B cell frequencies return to pre-infusion levels by the next infusion, maintaining a cyclic pattern of egress and return with each infusion cycle. **FIG. 7C** shows the returning B

5 cells exhibit increases in the co-stimulatory marker CD86, as well as the maturation marker CD95, suggesting that SL-172154 can induce phenotypic changes. Similarly, CD14+ monocytes marginate, or exit the circulation, within one hour of infusion; returning to pre-infusion levels by the next infusion, maintaining a cyclic pattern of egress and return for each infusion cycle. The observed pattern of margination is driven largely by CD86+ classical and non-classical monocytes (data not shown).

10 **FIG. 8A** and **FIG. 8B** show the distinct profile of TNF α and Interleukin-6 (IL-6) relative to CD40 mAbs. **FIG. 8A** shows the induction of TNF α at various doses of CP-870,893 (left panel) or SL-172154 (right panel). **FIG. 8B** shows the induction of IL-6 at various doses of CP-870,893 (left panel) or SL-172154 (right panel). The CP-870,893 data are from Vonderheide *et al.*, *J Clin Oncol* 25:876-883 (2007). These results demonstrate, *inter alia*, that SL-172154 does not induce TNF α and Interleukin-6 (IL-6) relative to CD40 mAbs. Dose limiting
15 toxicities (DLTs) have been attributed to cytokine release syndrome (CRS) have limited dose escalation of CD40 agonist mAbs. Interestingly, no notable increases in TNF α and IL-6 have been observed with SL-172154. As a consequence, SL-172154 is currently dosing at 10 $\times$ the dose of CP-870,893

FIG. 9A and **FIG. 9B** demonstrate that SL-172154 induces innate immune response in tumor microenvironment (TME). **FIG. 9A** shows the immunohistochemistry analysis of biopsy sample from patient
20 A before and after the administration of SL-172154. Monocytes were detected by staining for CD68 (a protein highly expressed by cells in the monocyte lineage). **FIG. 9B** demonstrates the upregulation of activation markers, CD40 and MHC Class II in TME in tumor biopsy sample after the treatment with SL-172154, compared to pretreatment biopsy samples.

FIG. 10A and **FIG. 10B** demonstrate that SL-172154 induces adaptive immune response in tumor
25 microenvironment (TME). **FIG. 9A** shows the CD8+ cells, Granzyme B+ cells, CD68+ cells, and Ki67+ cells in biopsy sample from patient A before and after the administration of SL-172154. CD8+ cells, Granzyme B+ cells, CD68+ cells, and Ki67+ cells increased in post-treatment biopsy sample, compared to pre-treatment biopsy sample. **FIG. 9B** is a plot comparing the tumor proportion score (TPS) and combined positive score (CPS). Induction of PD-L1 on immune cells is a consequence of CD8+ T cell activation.

30 These results suggest that SL-172154 was well-tolerated with no DLTs or evidence of anemia, thrombocytopenia, liver dysfunction, cytokine release syndrome or pneumonitis. Dose escalation continues at 10 mg/kg. Preliminary PK parameters for SL-172154 suggest target-mediated drug disposition via receptor binding. High receptor occupancy was observed for SL-172154 on CD47+ leukocytes at the doses studied, with minimal binding to RBCs. Binding of SL-172154 to CD40+ B cells and monocytes led to rapid activation

5 and margination post infusion. Cyclical increases in innate and adaptive serum cytokines were consistent with CD40 receptor engagement and activation. There were no increases in IL-6 or TNF α , nor evidence of bell-shaped dose responses. SL-172154 has been well-tolerated at doses which saturate both CD40 and CD47, with evidence of on-target PD activity which has not yet plateaued, warranting further dose escalation. These results further demonstrate, without wishing to be bound by theory, that SL-172154 induces innate as
10 well as adaptive immune response in tumor microenvironment (TME).

FIG. 11 shows the planned clinical development strategy for SL-172154. The strategy includes a trial for SL-172154 monotherapy in ovarian cancer, combination therapy of SL-172154 + liposomal doxorubicin in ovarian cancer, combination therapy of SL-172154 + azacitidine + venetoclax in AML, combination therapy of SL-172154 + azacitidine in HR-MDS, and combination therapy of SL-172154 + azacitidine in TP53 mutant
15 AML.

Example 2: Increased Expression of CD80 in Tumor Following the Administration of the SIRP α -Fc-CD40L Chimeric Protein (SL-172154)

SL-172154 was administered intratumorally to an ovarian cancer patient. A tumor biopsy sample was obtained from the patient prior to and after the administration of SL-172154. The biopsy samples were
20 analyzed for the expression of CD80 in the tumor. As shown in **FIG. 12**, compared to the biopsy sample obtained prior to the administration of SL-172154, the sample obtained after the administration of SL-172154 showed an increase in the abundance of CD80+ cells and/or CD80 expression in tumor following administration of SL-172154.

INCORPORATION BY REFERENCE

25 All patents and publications referenced herein are hereby incorporated by reference in their entireties.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior invention.

As used herein, all headings are simply for organization and are not intended to limit the disclosure in any
30 manner. The content of any individual section may be equally applicable to all sections.

EQUIVALENTS

While the invention has been disclosed in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or

5 adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation,
10 numerous equivalents to the specific embodiments disclosed specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

What is claimed is:

1. A method for treating a cancer in a human subject, the method comprising:
 - (i) administering to the human subject a first dose of a chimeric protein having a general structure of:
N terminus – (a) – (b) – (c) – C terminus,
wherein:
(a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),
(b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and
(c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L),
wherein a background level of a cell has been measured in a pre-dose biological sample obtained from the subject before the administration of the first dose, wherein the cell is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell;
 - (ii) administering to the human subject a second dose of the chimeric protein if a post-dosing level of the cell is greater than the background level of the cell,
wherein the second dose is administered at least about 48 hours after the administration of the first dose.
2. The method of claim 1, wherein the biological sample is selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen.
3. The method of claim 2, wherein the biological sample is a biopsy sample or a surgical specimen, optionally wherein the biological sample is a tumor biopsy sample or a tumor surgical specimen.
4. The method of claim 3, wherein the tumor biopsy sample or the tumor surgical specimen derived from a tumor selected ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN).

5. The method of any one of claims 1 to 4, wherein the level of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.
6. The method of claim 5, wherein the level of the cell is measured by contacting the sample with an agent that specifically binds to one or more of CD80, CD8, Granzyme B, CD68, Ki67 and PD-L1.
7. The method of claim 6, wherein the agent that specifically binds to the one or more molecules is an antibody or fragment thereof.
8. The method of claim 7, wherein the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof.
9. The method of claim 5, wherein the level of the cell is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding one or more of CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1.
10. The method of claim 9, wherein the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.
11. A method for treating a cancer in a human subject, the method comprising:
 - (i) administering to the human subject a first dose of a chimeric protein having a general structure of:
$$\text{N terminus} - (\text{a}) - (\text{b}) - (\text{c}) - \text{C terminus},$$
wherein:
 - (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),
 - (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and
 - (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L), wherein a background level and/or activity of B cells and/or CD40+ cells has been measured in a first biological sample obtained from the subject before the administration of the first dose; wherein an N-hr post-dose level and/or activity of B cells and/or CD40+ cells has been measured in a second biological sample obtained from the subject after the administration of the first dose, wherein N is a number between 1 and 24, wherein the N-hr post-dose level and/or

activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells;

wherein an M-day post-dose level and/or activity of B cells and/or CD40+ cells has been measured in a third biological sample obtained from the subject after the administration of the first dose, wherein M is a number between 1 and 28; and

(ii) administering to the human subject a second dose of the chimeric protein if the M-day post-dose level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells,

wherein the second dose is administered at least about 48 hours after the administration of the first dose.

12. The method of claim 11, wherein:
N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and/or
M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.
13. The method of claim 11, wherein:
N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and
M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.
14. The method of any one of claims 11 to 13, wherein the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is greater than the background level and/or activity of B cells and/or CD40+ cells.
15. The method of any one of claims 11 to 13, wherein the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells.
16. The method of any one of claims 11 to 13, wherein the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is within about 10%, or about 20%, or about 30%, or about 40% of the background level and/or activity of B cells and/or CD40+ cells.
17. The method of claim 11, wherein the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid,

tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen.

18. The method of claim 17, wherein the first biological sample, the second biological sample and the third biological sample are blood.

19. The method of any one of claims 11 to 18, wherein the level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

20. The method of claim 19, wherein the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to a CD40 and/or a B-cell marker.

21. The method of claim 20, wherein the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40.

22. The method of claim 20 or claim 21, wherein the agent that specifically binds to the one or more molecules is an antibody or fragment thereof.

23. The method of claim 22, wherein the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof.

24. The method of claim 19, wherein the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding a CD40 and/or a B-cell marker.

25. The method of claim 24, wherein the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40.

26. The method of claim 24 or claim 25, wherein the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

27. A method for treating a cancer in a human subject, the method comprising:

(i) administering to the human subject a first dose of a chimeric protein having a general structure of:

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

wherein:

(a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),

(b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and

(c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L), wherein a background amount and/or activity of a cytokine selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC has been measured in a first biological sample obtained from the subject before the administration of the first dose, and wherein an N-hr post-dose amount and/or activity of the cytokine has been measured in a second biological sample obtained from the subject after the administration of the first dose, wherein N is a number between 1 and 24, wherein the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine;

wherein an M-day post-dose amount and/or activity of the cytokine has been measured in a third biological sample obtained from the subject after the administration of the first dose, wherein M is a number between 1 and 28; and

(ii) administering to the human subject a second dose of the chimeric protein if the M-day post-dose amount and/or activity of the cytokine is at least about 30% lower than the N-hr post-dose amount and/or activity of the cytokine,

wherein the second dose is administered at least about 48 hours after the administration of the first dose.

28. The method of claim 27, wherein:

N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and/or

M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.

29. The method of claim 27, wherein:

N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and

M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.

30. The method of any one of claims 27 to 29, wherein the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine.
31. The method of any one of claims 27 to 29, wherein the N-hr post-dose amount and/or activity of the cytokine is less than the background amount and/or activity of the cytokine.
32. The method of any one of claims 27 to 29, wherein the N-hr post-dose amount and/or activity of the cytokine is within about 10%, or about 20%, or about 30%, or about 40% of the background amount and/or activity of the cytokine.
33. The method of claim 27, wherein the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen.
34. The method of claim 33, wherein the first biological sample, the second biological sample and the third biological sample are blood.
35. The method of any one of claims 27 to 34, wherein the amount and/or activity of the cytokine is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.
36. The method of claim 35, wherein the amount and/or activity of the cytokine is measured by contacting the sample with an agent that specifically binds to the cytokine.
37. The method of claim 36, wherein the agent that specifically binds to the one or more molecules is an antibody or fragment thereof, optionally wherein the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof.
38. The method of claim 35, wherein the amount and/or activity of the cytokine is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding the cytokine.
39. The method of claim 38, wherein the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

40. The method of any one of claims 1 to 39, wherein the second dose is administered at least about 3 days, or at least about 4 days, or at least about 5 days, or at least about 6 days, or at least about 7 days, or at least about 8 days, or at least about 9 days, or at least about 10 days, or at least about 14 days, or at least about 21 days, or at least about 28 days after the administration of the first dose,

41. A method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer, the method comprising:

- (i) obtaining a biological sample from the subject that has received a first dose of a chimeric protein;

wherein the chimeric protein has a general structure of:

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

wherein:

(a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),

(b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and

(c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L);

wherein a background level and/or activity of a cell has been measured in a biological sample obtained from the subject before the administration of the first dose, wherein the cell is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell;

- (ii) determining a post-dose level and/or activity of the cell in the biological sample; and
- (iii) determining that the chimeric protein is efficacious if the post-dose level and/or activity of the cell is greater than the background level and/or activity of the cell.

42. A method of selecting a subject for treatment with a therapy for a cancer, the method comprising:

- (i) obtaining a biological sample from the subject that has received a first dose of a chimeric protein;

wherein the chimeric protein has a general structure of:

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

wherein:

- (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),
- (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and
- (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L);

wherein a background level and/or activity of a cell has been measured in a biological sample obtained from the subject before the administration of the first dose, wherein the cell is selected from one or more of a CD80+ cell, a CD8+ cell, a Granzyme B+ cell, a CD68+ cell, a Ki67+ cell, and a PD-L1+ immune cell;

- (ii) determining a post-dose level and/or activity of the cell in the biological sample; and
- (iii) selecting the subject for treatment with the chimeric protein if the post-dose level and/or activity of the cell is greater than the background level and/or activity of the cell.

43. The method of claim 41 or claim 42, wherein the biological sample is selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen.

44. The method of claim 43, wherein the biological sample is a biopsy sample or a surgical specimen.

45. The method of claim 44, wherein the biological sample is a tumor biopsy sample or a tumor surgical specimen.

46. The method of claim 45, wherein the tumor biopsy sample or the tumor surgical specimen derived from a tumor selected ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN).

47. The method of any one of claims 41 to 46, wherein the level of the cell is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

48. The method of claim 47, wherein the level of the cell is measured by contacting the sample with an agent that specifically binds to one or more molecules selected from CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1.
49. The method of claim 48, wherein the agent that specifically binds to the one or more molecules is an antibody or fragment thereof.
50. The method of claim 49, wherein the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof.
51. The method of claim 47, wherein the level of the cell is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding one or more of CD80, CD8, Granzyme B, CD68, Ki67, and PD-L1.
52. The method of claim 51, wherein the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.
53. A method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer, the method comprising:
- (i) obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein;
wherein the chimeric protein has a general structure of:
$$\text{N terminus} - (a) - (b) - (c) - \text{C terminus},$$

wherein:
 - (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),
 - (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and
 - (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L);
 - (ii) determining a background level and/or activity of B cells and/or CD40+ cells in the first biological sample;
 - (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24, and determining an N-hr post-dose level and/or activity of B cells and/or CD40+ cells in the second biological sample;

- (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28, and determining an M-day post-dose level and/or activity of B cells and/or CD40+ cells in the third biological sample; and
 - (v) determining that the chimeric protein is efficacious if the N hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells, and/or if the M day post-dose level and/or activity of B cells and/or CD40+ cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells.
54. A method of selecting a subject for treatment with a therapy for a cancer, the method comprising:
- (i) obtaining a biological sample obtained from the subject that has received a first dose of a chimeric protein;
wherein the chimeric protein has a general structure of:
$$\text{N terminus} - (a) - (b) - (c) - \text{C terminus},$$

wherein:
 - (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),
 - (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and
 - (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L);
 - (ii) determining a background level and/or activity of B cells and/or CD40+ cells in the first biological sample;
 - (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24, and determining an N-hr post-dose level and/or activity of B cells and/or CD40+ cells in the second biological sample;
 - (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28, and determining an M-day post-dose level and/or activity of B cells and/or CD40+ cells in the third biological sample; and
 - (v) selecting the subject for treatment with the chimeric protein if the N hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells, and/or if the M day post-dose level and/or activity of B cells and/or CD40+

cells is at least about 50% higher than the N-hour post-dose level and/or activity of B cells and/or CD40+ cells.

55. The method of claim 53 or claim 54, wherein:
N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and/or
M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.
56. The method of claim 53 or claim 54, wherein:
N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and
M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.
57. The method of any one of claims 53 to 56, wherein the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is greater than the background level and/or activity of B cells and/or CD40+ cells.
58. The method of any one of claims 53 to 56, wherein the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is less than the background level and/or activity of B cells and/or CD40+ cells.
59. The method of any one of claims 53 to 56, wherein the N-hr post-dose level and/or activity of B cells and/or CD40+ cells is within about 10%, or about 20%, or about 30%, or about 40% of the background level and/or activity of B cells and/or CD40+ cells.
60. The method of claim 53 or claim 54, wherein the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen.
61. The method of claim 60, wherein the first biological sample, the second biological sample and the third biological sample are blood.

62. The method of any one of claims 53 to 61, wherein the level and/or activity of B cells and/or CD40+ cells is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.
63. The method of claim 62, wherein the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to a CD40 and/or a B-cell marker.
64. The method of claim 63, wherein the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40.
65. The method of claim 63 or claim 64, wherein the agent that specifically binds to the one or more molecules is an antibody or fragment thereof.
66. The method of claim 65, wherein the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof.
67. The method of claim 62, wherein the level and/or activity of B cells and/or CD40+ cells is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding a CD40 and/or a B-cell marker.
68. The method of claim 67, wherein the B-cell marker is selected from CD19, CD20, CD24, CD27, CD34, CD38, CD45R, CD86, CD95, IgM, IgD, and CD40.
69. The method of claim 67 or claim 68, wherein the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.
70. A method for evaluating the efficacy of cancer treatment in a subject in need thereof, wherein the subject is suffering from a cancer, the method comprising:
- (i) obtaining a first biological sample obtained from the subject that has received a first dose of a chimeric protein;
wherein the chimeric protein has a general structure of:
$$\text{N terminus} - (a) - (b) - (c) - \text{C terminus},$$

wherein:
 - (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),
 - (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and

- (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L);
- (ii) determining in the first biological sample a background amount and/or activity of a cytokine selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC;
 - (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24, and determining an N-hr post-dose amount and/or activity of the cytokine in the second biological sample;
 - (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28, and determining an M-day post-dose amount and/or activity of the cytokine in the third biological sample; and
 - (v) determining that the chimeric protein is efficacious if the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine, and/or if the M-day post-dose amount and/or activity of the cytokine is at least about 50% lower than the N-hour post-dose amount and/or activity of the cytokine.
71. A method of selecting a subject for treatment with a therapy for a cancer, the method comprising:
- (i) obtaining a biological sample obtained from the subject that has received a first dose of a chimeric protein;

wherein the chimeric protein has a general structure of:

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

wherein:

 - (a) is a first domain comprising an extracellular domain of human signal regulatory protein α (CD172a (SIRP α)),
 - (b) is a linker adjoining the first and second domains, wherein the linker comprises a hinge-CH2-CH3 Fc domain, and
 - (c) is a second domain comprising an extracellular domain of human CD40 ligand (CD40L);
 - (ii) determining in the first biological sample a background amount and/or activity of a cytokine selected from CCL2, CXCL9, CXCL10, IFN α , IL15, IL23, IL-12, MCP-1, MIP-1 β , MIP-1 α , and MDC;

- (iii) obtaining a second biological sample obtained from the subject at N hr post-dose, wherein N is a number between 1 and 24, and determining an N-hr post-dose amount and/or activity of the cytokine in the second biological sample;
 - (iv) obtaining a third biological sample obtained from the subject at M days post-dose, wherein M is a number between 1 and 28, and determining an M-day post-dose amount and/or activity of the cytokine in the third biological sample; and
 - (v) selecting the subject for treatment with the chimeric protein if the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine, and/or if the M-day post-dose amount and/or activity of the cytokine is at least about 50% lower than the N-hour post-dose amount and/or activity of the cytokine.
72. The method of claim 70 or claim 71, wherein:
N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and/or
M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.
73. The method of claim 70 or claim 71, wherein:
N is less than 12, or less than 8, or less than 6, or less than 4, or less than 3, or less than 2, or less than 1; and
M is less than 21, or less than 14, or less than 12, or less than 10, or less than 8, or less than 6, or less than 4, or less than 2.
74. The method of any one of claims 70 to 73, wherein the N-hr post-dose amount and/or activity of the cytokine is greater than the background amount and/or activity of the cytokine.
75. The method of any one of claims 70 to 73, wherein the N-hr post-dose amount and/or activity of the cytokine is less than the background amount and/or activity of the cytokine.
76. The method of any one of claims 70 to 73, wherein the N-hr post-dose amount and/or activity of the cytokine is within about 10%, or about 20%, or about 30%, or about 40% of the background amount and/or activity of the cytokine.
77. The method of claim 70 or claim 71, wherein the first biological sample, the second biological sample and the third biological sample are independently selected from blood, plasma, serum, blood cells, lacrimal

fluid, tears, bone marrow, ascites, tissue or fine needle biopsy sample, cell-containing body fluid, free floating nucleic acids, sputum, saliva, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, feces, lymph, gynecological fluid, skin swab, vaginal swab, oral swab, nasal swab, washing or lavage, aspirate, scraping, bone marrow specimen, a biopsy specimen and a surgical specimen.

78. The method of claim 77, wherein the first biological sample, the second biological sample and the third biological sample are blood.

79. The method of any one of claims 70 to 78, wherein the amount and/or activity of the cytokine is measured by RNA sequencing, immunohistochemical staining, western blotting, in cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS) or a combination thereof.

80. The method of claim 79, wherein the amount and/or activity of the cytokine is measured by contacting the sample with an agent that specifically binds to the cytokine.

81. The method of claim 80, wherein the agent that specifically binds to the one or more molecules is an antibody or fragment thereof.

82. The method of claim 81, wherein the antibody is a recombinant antibody, a monoclonal antibody, a polyclonal antibody, or fragment thereof.

83. The method of claim 79, wherein the amount and/or activity of the cytokine is measured by contacting the sample with an agent that specifically binds to one or more nucleic acids encoding the cytokine.

84. The method of claim 83, wherein the agent that specifically binds to one or more of the nucleic acids is a nucleic acid primer or probe.

85. The method of any one of claims 1 to 84, wherein the first dose of the chimeric protein is in the range of from about 0.03 mg/kg to 10 mg/kg.

86. The method of claim 85, wherein the first dose is about 0.003, or about 0.01, or about 0.03, or about 0.1, or about 0.3, or about 1, or about 2, or about 3, or about 4, or about 6, or about 8, or about 10 mg/kg.

87. The method of any one of claims 41 to 86, further comprising administration of a second dose of the chimeric protein.

88. The method of claim 87, wherein the second dose is administered at least about 3 days, or at least about 4 days, or at least about 5 days, or at least about 6 days, or at least about 7 days, or at least about 8

days, or at least about 9 days, or at least about 10 days, or at least about 14 days, or at least about 21 days, or at least about 28 days after the administration of the first dose.

89. The method of claim 87 or claim 88, wherein the second dose of the chimeric protein is in the range of from about 0.03 mg/kg to 10 mg/kg.

90. The method of any one of claims 87 to 89, wherein the second dose is about 0.003, or about 0.01, or about 0.03, or about 0.1, or about 0.3, or about 1, or about 2, or about 3, or about 4, or about 6, or about 8, or about 10 mg/kg.

91. The method of any one of claims 1 to 90, wherein the first domain is capable of binding a CD172a (SIRP α) ligand.

92. The method of any one of claims 1 to 91, wherein the first domain comprises substantially all of the extracellular domain of CD172a (SIRP α).

93. The method of any one of claims 1 to 92, wherein the second domain is capable of binding a CD40 receptor.

94. The method of any one of claims 1 to 93, wherein the second domain comprises substantially all of the extracellular domain of CD40L.

95. The method of any one of claims 1 to 94, wherein the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4.

96. The method of claim 95, wherein the linker comprises a hinge-CH2-CH3 Fc domain derived from human IgG4.

97. The method of any one of claims 1 to 96, wherein the linker comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

98. The method of any one of claims 1 to 97, wherein the first domain comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 57.

99. The method of any one of claims 1 to 98, wherein the second domain comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 58.
100. The method of any one of claims 1 to 99, wherein
- (a) the first domain comprises the amino acid sequence of SEQ ID NO: 57,
 - (b) the second domain comprises the amino acid sequence of SEQ ID NO: 58, and
 - (c) the linker comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.
101. The method of any one of claims 1 to 100, wherein the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7.
102. The method of any one of claims 1 to 101, wherein the chimeric protein further comprises the amino acid sequence of SEQ ID NO: 5 and SEQ ID NO: 7.
103. The method of any one of claims 1 to 102, wherein the chimeric protein comprises an amino acid sequence that is at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61.
104. The method of claim 103, wherein the chimeric protein comprises an amino acid sequence that is at least about 99% identical to the amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61.
105. The method of claim 103 or claim 104, wherein the chimeric protein comprises 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8 amino acid mutations with respect to an amino acid sequence of SEQ ID NO: 59 or SEQ ID NO: 61.
106. The method of any one of claims 1 to 105, wherein the human subject suffers from or is suspected to suffer from an advanced solid tumor or a lymphoma.
107. The method of any one of claims 1 to 106, wherein the human subject suffers from or is suspected to suffer from a cancer is selected from ovarian cancer, fallopian tube cancer, peritoneal cancer, cutaneous squamous cell carcinoma (CSCC), and squamous cell carcinoma of the head and neck (SCCHN).
108. The method of any one of claims 1 to 107, wherein the human subject has failed one or more platinum-based therapies.

109. The method of claim 108, wherein the human subject is ineligible for a further platinum therapy.
110. The method of any one of claims 1 to 108, wherein the human subject is ineligible for a platinum therapy.
111. The method of any one of claims 1 to 100, wherein the human subject is not receiving a concurrent chemotherapy, immunotherapy, biologic or hormonal therapy, and/or wherein the human subject has received, been tolerant to, or is ineligible for standard therapy and/or the cancer has no approved therapy considered to be standard of care.