

COMBINATION THERAPIES FOR TREATING CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of United States Provisional Application No. 63/188,388, filed on May 13, 2021, and of United States Provisional Application No. 63/193,581, filed on May 26, 2021, the contents of each of which are incorporated by reference in their entireties.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 757972001640SEQLIST.txt, date recorded: May 12, 2022, size: 302,942 bytes).

FIELD OF THE INVENTION

[0003] The present invention relates to methods of treating cancer that comprise administering an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) in conjunction with a chemotherapy agent and at least one additional anti-cancer agent and/or at least one additional mode of cancer therapy.

BACKGROUND

[0004] Many cancers have a poor prognosis, even when treated with available therapeutics. There is a need in the art for new treatments to provide additional therapeutic options and improve outcomes for cancer patients.

[0005] Tumor cells manipulate the myeloid compartment to evade the anti-tumor host immune response (Gabrilovich *et al.*, Nat Rev Immunol (2012) 12(4):253-68). For example, while CD47 expressed on the surface of normal cells binds SIRP α on macrophages and provides a “don’t eat me” signal, tumor cells have also been found to overexpress CD47 to evade the macrophage component of immune surveillance (Oldenborg, ISRN Hematol (2013) 614619).

[0006] Macrophage-mediated destruction of cancer cells requires both the disruption of “don’t eat me” signals (*e.g.*, CD47- SIRP α) and the activation of “eat me” signals. Neither component alone is sufficient to trigger maximal phagocytic reaction against tumor cells. As described above, CD47 provides a fundamental “don’t eat me” signal through its interaction with

SIRP α on macrophages. The pro-phagocytic “eat me” signal can be provided to the same macrophages by binding to their activating Fc gamma receptors. For example, the pro-phagocytic “eat me” signal can be provided by binding of anti-tumor antibodies to Fc receptors on macrophages.

[0007] All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0008] In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual an effective amount of: (a) a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant, (b) an anti-HER2 antibody, (c) an anti-VEGF2 antibody, and (d) paclitaxel; wherein the SIRP α D1 domain variant comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85; wherein the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat, wherein the cancer is gastric cancer or gastroesophageal junction (GEJ) cancer, and wherein the individual has received at least one prior therapy for the gastric or the GEJ cancer. In some embodiments, the gastric cancer or GEJ cancer is a HER2-overexpressing (*e.g.*, HER2⁺) gastric cancer or a HER2-overexpressing GEJ cancer. In some embodiments, the individual has received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, with an anti HER2 antibody and a platinum-based chemotherapy agent, or with a platinum-based chemotherapy agent. In some embodiments, individual has progressed during or after a treatment with an anti-HER2 antibody, an anti-HER2 antibody and a fluoropyrimidine, an anti HER2 antibody and a platinum-based chemotherapy agent, or a platinum-based chemotherapy agent. In some embodiments, the anti-

HER2 antibody is trastuzumab. In some embodiments, the anti-VEGF antibody is ramucirumab. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose between about 10 to about 60 mg/kg once a week (qw), such as 10 mg/kg, 15 mg/kg, or 30 mg/kg once a week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 10 mg/kg, 15 mg/kg, or 30 mg/kg once every two weeks. In some embodiments, the trastuzumab is administered at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks. In some embodiments, the trastuzumab is administered at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, an initial dose of 6 mg/kg, followed by a 4 mg/kg dose two weeks after the initial 6 mg/kg dose, followed by a 4 mg/kg dose every two weeks after the first 4 mg/kg dose). In some embodiments, the paclitaxel is administered at a dose of 80 mg/m² on days 1, 8, and 15 of a 28-day cycle. In some embodiments, among a population of patients receiving the treatment, the overall response rate (ORR) of the population is greater than 65%, 70%, 75%, 80%, 85%, 90%, or 95%.

[0009] In some embodiments, the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant forms a homodimer. In some embodiments, the individual is a human.

[0010] In some embodiments, provided is a kit comprising a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant in a pharmaceutically acceptable carrier, for use in combination with an anti-HER2 antibody, an anti-VEGFR2 antibody, and paclitaxel; wherein the SIRP α D1 domain variant comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85; wherein the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat, and wherein the kit comprises instructions for administering the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant in combination with the anti-HER2 antibody, the anti-VEGFR2 antibody, and the paclitaxel to an individual with gastric cancer or gastroesophageal junction (GEJ) cancer who has received at least one prior therapy for

the gastric or the GEJ cancer. In some embodiments, the gastric cancer or GEJ cancer is HER2⁺ gastric cancer or HER2⁺ GEJ cancer. In some embodiments, the anti-HER2 antibody is trastuzumab. In some embodiments, the anti-VEGFR2 antibody is ramucirumab. In some embodiments, the individual received prior therapy (or therapies) with an anti-HER2 antibody (*e.g.*, trastuzumab) and/or a fluoropyrimidine, and/or a platinum-based chemotherapeutic agent. In some embodiments, the gastric cancer or GEJ cancer in the individual progressed during or after prior therapy (or therapies) comprising anti-HER2 antibody (*e.g.*, trastuzumab) and/or a fluoropyrimidine, and/or a platinum-based chemotherapeutic agent. In some embodiments, the individual failed (*e.g.*, relapsed after or did not respond to) prior therapy (or therapies) comprising anti-HER2 antibody (*e.g.*, trastuzumab) and/or a fluoropyrimidine, and/or a platinum-based chemotherapeutic agent. In some embodiments, the prior therapy (or therapies) comprised an anti-HER2 antibody and a fluoropyrimidine (*e.g.*, administered during the same line of therapy or during different lines of therapy). In some embodiments, the prior therapy (or therapies) comprised an anti-HER2 antibody and a platinum-based chemotherapy agent (*e.g.*, administered during the same line of therapy or during different lines of therapy).

DESCRIPTION OF THE FIGURES

[0011] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0012] **FIG 1** provides the results of experiments that were performed to determine the effect of DRUG A in combination with (a) an anti-HER2 antibody, (b) an anti-PD-L1 antibody, or (c) an anti-HER2 antibody and an anti-PD-L1 on tumor growth in a MC38 m.h chimeric colon cancer model in mice.

[0013] **FIG 2A** provides a graph of the best % change in baseline measurable lesions in each patient in the study described in Example 2E.

[0014] **FIG 2B** provides a graph showing the % change in baseline measurable lesions in each patient in the study described in Example 2E as a function of time .

[0015] **FIG 3** shows the results of experiments that were performed to assess whether DRUG B enhances the anti-tumor activity of anti-mouse VEGFR-2 + paclitaxel doublet therapy in a CT26

syngeneic mouse tumor model.

[0016] **FIG 4A** shows the results of experiments that were performed to assess whether DRUG B enhances the anti-tumor activity of trastuzumab + anti-mouse VEGFR-2 + paclitaxel triplet therapy in a CT26 m:h HER2-expression chimeric tumor model in mice.

[0017] **FIG 4B** shows the results of experiments that were performed to assess whether quadruplet therapy with DRUG B + trastuzumab + anti-mouse VEGFR-2 + paclitaxel increases the probability of survival compared to triplet therapy with trastuzumab + anti-mouse VEGFR-2 + paclitaxel in a CT26 m:h HER2-expression chimeric tumor model in mice.

DETAILED DESCRIPTION

[0018] The following description sets forth exemplary methods, parameters and the like. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure but is instead provided as a description of exemplary embodiments.

Definitions

[0019] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0020] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description or the claims, such terms are intended to be inclusive in

a manner similar to the term “comprising.”

[0021] As used herein, the terms “treatment”, “treating”, and the like, refer to administering an agent, or carrying out a procedure, for the purposes of obtaining an effect. In some embodiments, the effect is prophylactic in terms of completely or partially preventing a disease or symptom thereof. In some embodiments, the effect is therapeutic in terms of affecting a partial or complete cure for a disease or symptoms of the disease.

[0022] As used herein, the term “antibody” refers to intact antibodies; antibody fragments, provided that they exhibit the desired biological activity (*e.g.* epitope binding); monoclonal antibodies; polyclonal antibodies; monospecific antibodies; multi-specific antibodies (*e.g.*, bispecific antibodies); and antibody-like proteins.

[0023] As used herein, the term “antibody variable domain” refers to the portions of the light and heavy chains of an antibody that include amino acid sequences of complementary determining regions (CDRs, *e.g.*, CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, and CDR H3) and framework regions (FRs).

[0024] As used herein, the term “linker” refers to a linkage between two elements, *e.g.*, protein domains. In some embodiments, a linker can be a covalent bond or a spacer. The term “spacer” refers to a moiety (*e.g.*, a polyethylene glycol (PEG) polymer) or an amino acid sequence (*e.g.*, a 1-200 amino acid sequence) occurring between two polypeptides or polypeptide domains to provide space or flexibility (or both space and flexibility) between the two polypeptides or polypeptide domains. In some embodiments, an amino acid spacer is part of the primary sequence of a polypeptide (*e.g.*, joined to the spaced polypeptides or polypeptide domains via the polypeptide backbone).

[0025] As used herein, the term “effective amount” refers to an amount of a polypeptide or a pharmaceutical composition containing a polypeptide described herein, *e.g.*, a polypeptide having a SIRPα D1 domain or variant thereof, that is sufficient and effective in achieving a desired therapeutic effect in treating a patient having a disease, such as a cancer, *e.g.*, solid tumor or hematological cancer. In some embodiments, an effective amount of polypeptide will avoid adverse side effects.

[0026] As used herein, the term “pharmaceutical composition” refers to a medicinal or

pharmaceutical formulation that includes an active ingredient as well as excipients or diluents (or both excipients and diluents) and enables the active ingredient to be administered by suitable methods of administration. In some embodiments, the pharmaceutical compositions disclosed herein include pharmaceutically acceptable components that are compatible with the polypeptide. In some embodiments, the pharmaceutical composition is in tablet or capsule form for oral administration or in aqueous form for intravenous or subcutaneous administration, for example by injection.

[0027] As used herein, the terms “subject,” “individual,” and “patient” are used interchangeably to refer to a vertebrate, for example, a mammal. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells, and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed. None of the terms entail supervision of a medical professional.

[0028] As used herein, the term “affinity” or “binding affinity” refers to the strength of the binding interaction between two molecules. Generally, binding affinity refers to the strength of the sum total of non-covalent interactions between a molecule and its binding partner, such as a SIRP α D1 domain variant and CD47. Unless indicated otherwise, binding affinity refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair. The binding affinity between two molecules is commonly described by the dissociation constant (K_D) or the association constant (K_A). Two molecules that have low binding affinity for each other generally bind slowly, tend to dissociate easily, and exhibit a large K_D. Two molecules that have high affinity for each other generally bind readily, tend to remain bound longer, and exhibit a small K_D. In some embodiments, the K_D of two interacting molecules is determined using known methods and techniques, *e.g.*, surface plasmon resonance (SPR). K_D can be calculated as the ratio of k_{off}/k_{on} .

[0029] As used herein, the term “K_D less than” refers to a numerically smaller K_D value and an increasing binding affinity relative to the recited K_D value. As used herein, the term “K_D greater than” refers to a numerically larger K_D value and a decreasing binding affinity relative to the recited K_D value.

[0030] As used herein, “in conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” refers to

administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

Overview

[0031] Provided herein are methods of treating cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) and (b) a chemotherapy agent (*e.g.*, at least one chemotherapy agent, such as at least two, at least three, or at least four chemotherapy agents). In some embodiments the method further comprises administering to the individual an effective amount of a therapeutic antibody (*e.g.*, at least one therapeutic antibody, such as at least two, at least three, or at least four therapeutic antibodies). Additionally or alternatively, in some embodiments the method further comprises administering to the individual an effective amount of an immunotherapeutic agent (*e.g.*, at least one immunotherapeutic agent, such as at least two, at least three, or at least four immunotherapeutic agents). Additionally or alternatively, in some embodiments, the method comprises administering the polypeptide and the chemotherapy agent in combination with one or more additional modes of therapy, including, but not limited to, *e.g.*, radiation therapy, surgery, cryoablation, and bone marrow transplant.

[0032] In some embodiments, the agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) is a small molecule inhibitor of the CD47-SIRP α pathway (*e.g.*, RRX-001 and others). See, *e.g.*, Miller *et al.* (2019) "Quantitative high-throughput screening assays for the discovery and development of SIRP α -CD47 interaction inhibitors." *PLoS ONE* 14(7): e0218897 and Sasikumar *et al.* ACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; October 26-30, 2017; Philadelphia, PA; Abstract B007.

[0033] In some embodiments, the agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) binds CD47 (*e.g.*, hCD47). In some embodiments, the agent binds CD47 (*e.g.*, hCD47) with a K_D of about 10 nM or better (such as at least about any one of 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 3 nM, 2 nM, 1 nM, 750 pM, 500 pM, 250 pM, 200 pM, 100 pM, 50 pM, 25 pM, 20 pM, 10 pM or less than 10 pM). In some embodiments, the agent that binds CD47

(*e.g.*, hCD47) exhibits at least about 50% CD47 receptor occupancy (*e.g.*, at least about any one of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or about 100%) in a human subject. In some embodiments, the agent that binds CD47 (*e.g.*, hCD47) has an EC₅₀ of about 80 ng/ml or less, *e.g.*, about any one of 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 ng/ml. In some embodiments, the agent that binds CD47 (*e.g.*, hCD47) is an anti-CD47 antibody (*e.g.*, a therapeutic anti-CD47 antibody) or an antigen-binding fragment thereof. In some embodiments, the antigen binding fragment is a Fab, a Fab', a Fab'-SH, an F(ab')₂, an Fv, an scFv, a one-armed antibody, or a diabody. In some embodiments, the anti-CD47 antibody is a monospecific antibody. In some embodiments, the anti-CD47 antibody is a multispecific (*e.g.*, bispecific) antibody. In some embodiments the term "anti-CD47 antibody" encompasses antibody-based constructs (such as multispecific constructs) including, without limitation triomabs, DARTs (*i.e.*, dual-affinity re-targeting antibodies), TandAbs (*i.e.*, tandem diabodies), tandem scFvs, CrossMabs, DNLs (*i.e.*, dock and lock antibodies), DVD-Ig (*i.e.*, dual variable domain immunoglobulins), tetravalent bispecific IgGs, nanobodies, dual targeting domains, and ART-Igs (*i.e.*, asymmetric reengineering technology-immunoglobulins). Additional details regarding exemplary antibody constructs (both monospecific and multispecific) are provided in Husain *et al.* (2018) *Biodrugs* 32(5): 441-464 and Spiess *et al.* (2015) *Molecular Immunology* 67(2): 95-106. In some embodiments, the anti-CD47 antibody is Hu5F9-G4, B6H12.2, BRIC126, CC-90002, SRF231, or IBI188 (from Innovent Biologics) (*see, e.g.*, Zhao *et al.* (2011), *PNAS USA* 108:18342-18347; Chao *et al.* (2010) *Cell* 142:699-713, Kim *et al.* (2012) *Leukemia* 26:2538-2545; Chao *et al.* (2011) *Blood* 118:4890-4891; Goto *et al.* (2014) *Eur J. Cancer* 50:1836-1846; and Edris *et al.* (2012) *PNAS USA* 109:6656-61 for additional information about these anti-CD47 antibodies).

[0034] In some embodiments, the agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRPα (*e.g.*, hSIRPα) binds SIRPα (*e.g.*, hSIRPα). In some embodiments, the agent binds SIRPα (*e.g.*, hSIRPα) with a K_D of about 10 nM or better (such as at least about any one of 9nM, 8 nM, 7nM, 6nM, 5nM, 3nM, 2nM, 1nM, 750 pM, 500pM, 250pM, 200pM, 100pM, 50 pM, 25 pM, 20 pM 10pM or less than 10 pM). In some embodiments, the agent that binds SIRPα (*e.g.*, hSIRPα) exhibits at least about 50% SIRPα receptor occupancy (*e.g.*, at least about any one of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or about 100%) in a human subject. In some embodiments, the agent that binds SIRPα (*e.g.*, hSIRPα) has an EC₅₀ of

about 80 ng/ml or less, *e.g.*, about any one of 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 ng/ml. In some embodiments, the agent that binds SIRP α (*e.g.*, hSIRP α) is an anti-SIRP α antibody (*e.g.*, a therapeutic anti-SIRP α antibody) or an antigen-binding fragment thereof. In some embodiments, the antigen binding fragment is a Fab, a Fab', a Fab'-SH, an F(ab')₂, an Fv, an scFv, a one-armed antibody, or a diabody. In some embodiments, the anti-SIRP α antibody is a monospecific antibody or monospecific antibody construct (including, but not limited to those described above). In some embodiments, the anti-SIRP α antibody is a multispecific (*e.g.*, bispecific) antibody or a multispecific antibody construct (including, but not limited to those described above). In some embodiments, the anti-SIRP α antibody is KWAR23, SE12C3, 040, or MY-1 (see, *e.g.*, Ring et al. (2017) *PNAS USA* 114(49): E10578-E10585; Murata et al. (2018) *Cancer Sci* 109(5):1300-1308; and Yanigata et al. (2017) *JCI Insight* 2:e89140 for additional information about these anti-SIRP α antibodies). In some embodiments, the anti-SIRP α antibody is an antibody described in WO 2018/057669; US-2018-0105600-A1; US20180312587; WO2018107058; WO2019023347; US20180037652; WO2018210795; WO2017178653; WO2018149938; WO2017068164; and WO2016063233, the contents of which are incorporated herein by reference in their entireties.

[0035] In some embodiments, the agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) is an anti-SIRP β antibody or an anti-SIRP γ antibody (*e.g.*, an anti-SIRP β antibody or anti-SIRP γ antibody that is capable of binding SIRP α), or an antigen-binding fragment thereof. In some embodiments, the agent is an antibody (or antigen binding fragment thereof) that is capable of bind two or more of SIRP α , SIRP β , and SIRP γ . In some embodiments, such antibody binds SIRP α (*e.g.*, hSIRP α) with a K_D of about 10 nM or better (such as at least about any one of 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 3nM, 2 nM, 1 nM, 750 pM, 500 pM, 250 pM, 200 pM, 100 pM, 50 pM, 25 pM, 20 pM, 10 pM or less than 10 pM). In some embodiments, the antibody exhibits at least about 50% SIRP α receptor occupancy (*e.g.*, at least about any one of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or about 100%) in a human subject. In some embodiments, the antibody has an EC₅₀ of about 80 ng/ml or less, *e.g.*, about any one of 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 ng/ml. In some embodiments, the antigen binding fragment is a Fab, a Fab', a Fab'-SH, an F(ab')₂, an Fv, an scFv, a one-armed antibody, or a diabody. In some embodiments, the antibody is a monospecific antibody or monospecific antibody construct (including, but not limited to those described

above). In some embodiments, the antibody is a multispecific (*e.g.*, bispecific) antibody or a multispecific antibody construct (including, but not limited to those described above).

[0036] In some embodiments, the agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) is a fusion polypeptide comprising a moiety that binds CD47. In some embodiments, the fusion polypeptide comprises an antibody Fc region and a moiety that binds CD47. In some embodiments, the portion of the fusion polypeptide that binds CD47 (*e.g.*, hCD47) binds CD47 (*e.g.*, hCD47) with a K_D of about 10 nM or better (such as at least about any one of 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 3 nM, 2 nM, 1 nM, 750 pM, 500 pM, 250 pM, 200 pM, 100 pM, 50 pM, 25 pM, 20 pM, 10 pM or less than 10 pM). In some embodiments, the fusion polypeptide exhibits at least about 50% CD47 receptor occupancy (*e.g.*, at least about any one of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or about 100%) in a human subject. In some embodiments, the fusion polypeptide has an EC₅₀ of about 80 ng/ml or less, *e.g.*, about any one of 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 ng/ml. In some embodiments, the fusion polypeptide comprises WT human antibody Fc region. In some embodiments, the fusion polypeptide comprises an Fc variant (*e.g.*, a variant of a WT human antibody Fc region) that exhibits reduced (*e.g.*, such as ablated) effector function as compared to a WT Fc region. Exemplary Fc variants are described in WO 2017/027422 and US 2017/0107270, the contents of which are incorporated herein by reference in their entireties. In some embodiments, moiety that binds CD47 (*e.g.*, hCD47) is a WT SIRP α (*e.g.*, hSIRP α), or a WT SIRP γ (*e.g.*, hSIRP γ). In some embodiments, moiety that binds CD47 (*e.g.*, hCD47) is a CD47-binding fragment (*e.g.*, d1 domain) of a WT SIRP α (*e.g.*, hSIRP α), or a WT SIRP γ (*e.g.*, hSIRP γ). In some embodiments, the moiety that binds CD47 (*e.g.*, hCD47) is a SIRP α variant, a SIRP γ variant, a SIRP β variant, or a CD47-binding fragment thereof (*e.g.*, the d1 domain). Exemplary SIRP γ variants, SIRP β 1 variant, and SIRP β 2 variants are described in, *e.g.*, WO 2013/109752; US 2015/0071905; USP 9,944,911; WO 2016/023040; WO 2017/027422; US 2017/0107270; USP 10,259,859; US9845345; WO2016187226; US20180155405; WO2017177333; WO2014094122; US2015329616; US20180312563; WO2018176132; WO2018081898; WO2018081897; PCT/US2019/048921; US20180141986A1; and EP3287470A1, the contents of which are incorporated herein by reference in their entireties.

[0037] In some embodiments, the agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) is a fusion polypeptide comprising an antibody Fc region and

a SIRP α variant. In some embodiments, the SIRP α variant binds CD47 (*e.g.*, hCD47) with a K_D of about 10 nM or better (such as at least about any one of 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 3 nM, 2 nM, 1 nM, 750 pM, 500 pM, 250 pM, 200 pM, 100 pM, 50 pM, 25 pM, 20 pM, 10 pM or less than 10 pM). In some embodiments, the fusion polypeptide exhibits at least about 50% CD47 receptor occupancy (*e.g.*, at least about any one of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or about 100%) in a human subject. In some embodiments, the fusion polypeptide has an EC₅₀ of about 80 ng/ml or less, *e.g.*, about any one of 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 ng/ml. In some embodiments, the fusion polypeptide comprises WT human antibody Fc region. In some embodiments, the fusion polypeptide comprises an Fc variant (*e.g.*, a variant of a WT human antibody Fc region) that exhibits reduced (*e.g.*, such as ablated) effector function as compared to a WT Fc region, such as those described in the references cited herein. In some embodiments, the fusion polypeptide comprises a SIRP α variant described in WO 2013/109752; US 2015/0071905; WO 2016/023040; WO 2017/027422; US 2017/0107270; USP 10,259,859; US9845345; WO2016187226; US20180155405; WO2017177333; WO2014094122; US2015329616; US20180312563; WO2018176132; WO2018081898; WO2018081897; US20180141986A1; and EP3287470A1, the contents of which are incorporated herein by reference in their entireties. In some embodiments, the fusion polypeptide comprising an antibody Fc region and a SIRP α variant is TTI-621, TTI-622, or IMM01 (see, *e.g.*, Petrova et al. (2017) Clin Cancer Res 23:1086-1079; Russ et al. (2018) Blood Rev S0268-960X(17)30093-0; Zhang, X, Chen, W, Fan, J et al. Disrupting CD47-SIRP α axis alone or combined with autophagy depletion for the therapy of glioblastoma. Carcinogenesis 2018; 39: 689–99).

[0038] In some embodiments, the agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) is a fusion polypeptide comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein).

[0039] In some embodiments, provided is a method of treating cancer (*e.g.*, gastric cancer or gastroesophageal cancer) in an individual (*e.g.*, a human individual), comprising administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) (b) an anti-HER2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel. In some embodiments, the agent that blocks the interaction between CD47

and SIRP α is a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant) wherein the SIRP α D1 domain variant comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85, wherein the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat.

[0040] Further details regarding the methods of treatment with polypeptides comprising a SIRP α D1 domain variant and an Fc domain variant are described below. See also WO 2017/027422, US Patent No. 10,259,859, and PCT/US20/62402 the contents of each of which are incorporated by reference herein in their entireties.

Signal-Regulatory Protein α (SIRP α) D1 Domain and Variants Thereof

[0041] Disclosed herein, in some embodiments, are polypeptides comprising a signal-regulatory protein α (SIRP- α) D1 variant comprising a SIRP α D1 domain, or a fragment thereof, that comprises an amino acid mutation at residue 80 relative to a wild-type SIRP α D1 domain (e.g., a wild-type SIRP α D1 domain set forth in SEQ ID NO: 1 or 2); and at least one additional amino acid mutation relative to a wild-type SIRP α D1 domain (e.g., a wild-type SIRP α D1 domain set forth in SEQ ID NO: 1 or 2) at a residue selected from the group consisting of: residue 6, residue 27, residue 31, residue 47, residue 53, residue 54, residue 56, residue 66, and residue 92.

[0042] Also disclosed herein, in some embodiments, are polypeptides comprising an Fc domain variants, wherein an Fc domain variant dimer comprises two Fc domain variants, wherein each Fc domain variant independently is selected from (i) a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A; (ii) a human IgG2 Fc region consisting of mutations A330S, P331S and N297A; or (iii) a human IgG4 Fc region comprising mutations S228P, E233P, F234V, L235A, delG236, and N297A.

[0043] Signal-regulatory protein α (“SIRP- α ” or “SIRP-alpha”) is a transmembrane glycoprotein belonging to the Ig superfamily that is widely expressed on the membrane of myeloid cells. SIRP α interacts with CD47, a protein broadly expressed on many cell types in the body. The interaction of SIRP α with CD47 prevents engulfment of “self” cells, which can otherwise be recognized by the immune system. It has been observed that high CD47 expression on tumor cells can act, in acute myeloid leukemia and several solid tumor cancers, as a negative prognostic factor for survival.

[0044] Native SIRP α comprises 3 highly homologous immunoglobulin (Ig)-like extracellular domains—D1, D2, and D3. The SIRP α D1 domain (“D1 domain”) refers to the membrane distal, extracellular domain of SIRP α and mediates binding of SIRP α to CD47. As used herein, the term “SIRP α polypeptide” refers to any SIRP α polypeptide or fragment thereof that is capable of binding to CD47. There are at least ten variants of wild-type human SIRP α . *Table 1* shows the amino acid sequences of the D1 domains of the naturally occurring wild-type human SIRP α D1 domain variants (SEQ ID NOs: 1 and 2). In some embodiments, a SIRP α polypeptide comprises a SIRP α D1 domain. In some embodiments, a SIRP α polypeptide comprises a wild-type D1 domain, such as those provided in SEQ ID NOs: 1 and 2. In some embodiments, a SIRP α polypeptide includes a D2 or D3 domain (or both a D2 and a D3 domain) (see Table 3) of a wild-type human SIRP α .

Table 1. Sequences of Wild-Type SIRPα D1 Domains

SEQ ID NO:	Description	Amino Acid Sequence
1	Wild-type D1 domain variant 1	EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQ WFRGAGPGRELIYNQKEGHFPRVTTVSDLTKRNNM DFSIRIGNITPADAGTY YCVKFRKGS PDDVEFKSGA GTELSVR AKPS
2	Wild-type D1 domain variant 2	EEELQVIQPDKSVSVAAGESAILHCTVTSLIPVGPIQ WFRGAGPARELIYNQKEGHFPRVTTVSESTKRENM DFSISISNITPADAGTY YCVKFRKGS PDTEFKSGAGT ELSVRAKPS
11	Wild-type pan-D1 domain	EEX ₁ LQVIQPDKX ₂ VX ₃ VAAGEX ₄ AX ₅ LX ₆ CTX ₇ TS LIPVGPIQWFRGAGPX ₈ RELIYNQKEGHFPRVTTVSX ₉ X ₁₀ TKRX ₁₁ NMDFX ₁₂ IX ₁₃ IX ₁₄ NITPADAGTY YCVKFRKGS X ₁₅ X ₁₆ DX ₁₇ EFKSGAGTELSVRX ₁₈ KPS
	Amino acid substitutions relative to SEQ ID NO: 11	X ₁ is E or G; X ₂ is S or F; X ₃ is L or S; X ₄ is T or S; X ₅ is T or I; X ₆ is R, H, or L; X ₇ is A or V; X ₈ is G or A; X ₉ is D or E; X ₁₀ is L or S; X ₁₁ is N or E or D; X ₁₂ is S or P; X ₁₃ is R or S; X ₁₄ is G or S; X ₁₅ is P or absent; X ₁₆ is D or P; X ₁₇ is V or T; and X ₁₈ is A or G

[0045] As used herein, the term “SIRPα D1 domain variant” refers to a polypeptide comprising a SIRPα D1 domain or a CD47-binding portion of a SIRPα polypeptide that has a higher affinity to CD47 than wild-type SIRPα. A SIRPα D1 domain variant comprises at least one amino acid substitution, deletion, or insertion (or a combination thereof) relative to a wild-type SIRPα.

[0046] In some embodiments, SIRPα D1 domain variants disclosed herein comprise a SIRPα D1 domain or variant thereof. In some embodiments, a SIRPα D1 domain variant comprises one or more amino acid substitutions, insertions, additions, or deletions relative to a wild-type D1 domain shown in SEQ ID NOs: 1 and 2. **Table 2** lists exemplary amino acid substitutions in each SIRPα D1 domain variant (SEQ ID NOs: 13-14). In some embodiments, the SIRPα D1 domain polypeptide or SIRPα D1 domain variant comprises a fragment of the D1 domain. In some embodiments, the SIRPα polypeptide fragment or SIRPα D1 domain variant fragment comprises an amino acid sequence of less than 10 amino acids in length, about 10 amino acids in length, about 20 amino acids in length, about 30 amino acids in length, about 40 amino acids in length, about 50 amino acids in length, about 60 amino acids in length, about 70 amino acids in length,

about 80 amino acids in length, about 90 amino acids in length, about 100 amino acids in length, or more than about 100 amino acids in length. In some embodiments, the SIRP α D1 domain fragments retain the ability to bind to CD47.

[0047] In some embodiments, a polypeptide of the disclosure comprising a SIRP α D1 domain variant binds with higher binding affinity to CD47 than a wild-type human SIRP α D1 domain. In some embodiments, the SIRP α D1 domain variant binds to human CD47 with at least 1-fold (*e.g.*, at least 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 5-fold or greater than 5-fold) affinity than the affinity of a naturally occurring D1 domain. In some embodiments, the SIRP α D1 domain variant binds to human CD47 with at least 1-fold (*e.g.*, at least 10-fold, 100-fold, 1000-fold or greater than 1000-fold) affinity than the affinity of a naturally occurring D1 domain.

[0048] As used herein, the term “optimized affinity” or “optimized binding affinity” refers to an optimized strength of the binding interaction between a polypeptide disclosed herein, including a SIRP α D1 domain variant, and CD47. For example, in some embodiments, the polypeptide binds primarily or with higher affinity to CD47 on cancer cells and does not substantially bind or binds with lower affinity to CD47 on non-cancer cells. In some embodiments, the binding affinity between the polypeptide and CD47 is optimized such that the interaction does not cause clinically relevant toxicity or decreases toxicity compared to a variant which binds with maximal affinity. In some embodiments, in order to achieve an optimized binding affinity between a polypeptide provided herein and CD47, the polypeptide including a SIRP α D1 domain variant is developed to have a lower binding affinity to CD47 than which is maximally achievable. In some embodiments, the SIRP α D1 domain variants disclosed herein cross react with rodent, non-human primate (NHP), and human CD47.

[0049] As used herein, the term “immunogenicity” refers to the property of a protein (*e.g.*, a therapeutic protein) which causes an immune response in the host as though it is a foreign antigen. The immunogenicity of a protein can be assayed *in vitro* in a variety of different ways, such as through *in vitro* T-cell proliferation assays.

[0050] As used herein, the term “minimal immunogenicity” refers to an immunogenicity of a protein (*e.g.*, a therapeutic protein) that has been modified, *e.g.*, through amino acid substitutions, to be lower (*e.g.*, at least 10%, 25%, 50%, or 100% lower) than the

immunogenicity before the amino acid substitutions are introduced (*e.g.*, an unmodified protein). In some embodiments, a protein (*e.g.*, a therapeutic protein) is modified to have minimal immunogenicity and causes no or very little host immune response even though it is a foreign antigen.

[0051] In some embodiments, the SIRP α D1 domain variant demonstrates minimal immunogenicity. In some embodiments, a SIRP α polypeptide of the disclosure administered to a subject has the same amino acid sequence as that of the SIRP α polypeptide in a biological sample of the subject, except for amino acid changes which increase affinity of the SIRP α D1 domain variant. In some embodiments, the polypeptide variants disclosed herein lower the risk of side effects compared to anti-CD47 antibodies or wild-type SIRP α . In some embodiments, the polypeptide variants disclosed herein lower the risk of anemia compared to anti-CD47 antibodies or wild-type SIRP α . In some embodiments, the polypeptide variants disclosed herein do not cause acute anemia in rodent or non-human primates (NHP) studies.

[0052] *Table 2* lists specific amino acid substitutions in a SIRP α D1 domain variant relative to each D1 domain sequence. In some embodiments, a SIRP α D1 domain variant includes one or more (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or more) of the substitutions listed in *Table 2*. In some embodiments, a SIRP α D1 domain variant includes at most fourteen amino acid substitutions relative to a wild-type D1 domain. In some embodiments, a SIRP α D1 domain variant includes at most ten amino acid substitutions relative to a wild-type D1 domain. In some embodiments, a SIRP α D1 domain variant includes at most seven amino acid substitutions relative to a wild-type D1 domain. In some embodiments, a SIRP α D1 domain variant of the disclosure has at least 90% (*e.g.*, at least 92%, 95%, 97% or greater than 97%) amino acid sequence identity to a sequence of a wild-type D1 domain.

[0053] In some embodiments, a SIRP α D1 domain variant is a chimeric SIRP α D1 domain variant that includes a portion of two or more wild-type D1 domains or variants thereof (*e.g.*, a portion of one wild-type D1 domain or variant thereof and a portion of another wild-type D1 domain or variant thereof). In some embodiments, a chimeric SIRP α D1 domain variant includes at least two portions (*e.g.*, three, four, five or more portions) of wild-type D1 domains or variants thereof, wherein each of the portions is from a different wild-type D1 domain. In some embodiments, a chimeric SIRP α D1 domain variant further includes one or more amino acid

substitutions listed in *Table 2*.

Table 2. Amino Acid Substitutions in a SIRPα D1 Domain Variant

SEQ ID NO:	Description	Amino Acid Sequence
13	D1 domain v1	EEEX ₁ QX ₂ IQPDKSVLVAAGETX ₃ TLRCTX ₄ TS LX ₅ PVGPIQWFRGAGPGRX ₆ LIYNQX ₇ X ₈ GX ₉ FPRVTTVSDX ₁₀ TX ₁₁ RNNMDFSIRIGNITPADAGTYCYX ₁₂ KX ₁₃ RKGSPDDVEX ₁₄ KSGAGTELSVRAKPS
-	Amino acid substitutions relative to SEQ ID NO: 13	X ₁ =L, I, V; X ₂ =V, L, I; X ₃ =A, V; X ₄ =A, I, L; X ₅ =I, T, S, F; X ₆ =E, V, L; X ₇ =K, R; X ₈ =E, Q; X ₉ =H, P, R; X ₁₀ =L, T, G; X ₁₁ =K, R; X ₁₂ =V, I; X ₁₃ =F, L, V; X ₁₄ =F, V
14	D1 domain v2	EEEX ₁ QX ₂ IQPDKSVSVAAGESX ₃ ILHCTX ₄ TS LX ₅ PVGPIQWFRGAGPARX ₆ LIYNQX ₇ X ₈ GX ₉ FPRVTTVSEX ₁₀ TX ₁₁ RENMDFSISISNITPADAGTYCYX ₁₂ KX ₁₃ RKGSPDTEX ₁₄ KSGAGTELSVRAKPS
-	Amino acid substitutions relative to SEQ ID NO: 14	X ₁ =L, I, V; X ₂ =V, L, I; X ₃ =A, V; X ₄ =V, I, L; X ₅ =I, T, S, F; X ₆ =E, V, L; X ₇ =K, R; X ₈ =E, Q; X ₉ =H, P, R; X ₁₀ =S, T, G; X ₁₁ =K, R; X ₁₂ =V, I; X ₁₃ =F, L, V; X ₁₄ =F, V
23	Pan D1 domain	EEX ₁ X ₂ QX ₃ IQPDKX ₄ VX ₅ VAAGEX ₆ X ₇ X ₈ LX ₉ CTX ₁₀ TS LX ₁₁ PVGPIQWFRGAGPX ₁₂ RX ₁₃ LIYNQX ₁₄ X ₁₅ GX ₁₆ FPRVTTVSX ₁₇ X ₁₈ TX ₁₉ RX ₂₀ NMDFX ₂₁ IX ₂₂ IX ₂₃ NITPADAGTYCYX ₂₄ KX ₂₅ RKGSPDX ₂₆ X ₂₇ EX ₂₈ KSGAGTELSVRX ₂₉ KPS
-	Amino acid substitutions relative to SEQ ID NO: 23	X ₁ =E, G; X ₂ =L, I, V; X ₃ =V, L, I; X ₄ =S, F; X ₅ =L, S; X ₆ =S, T; X ₇ =A, V; X ₈ =I, T; X ₉ =H, R; X ₁₀ =A, V, I, L; X ₁₁ =I, T, S, F; X ₁₂ =A, G; X ₁₃ =E, V, L; X ₁₄ =K, R; X ₁₅ =E, Q; X ₁₆ =H, P, R; X ₁₇ =D, E; X ₁₈ =S, L, T, G; X ₁₉ =K, R; X ₂₀ =E, D; X ₂₁ =S, P; X ₂₂ =S, R; X ₂₃ =S, G; X ₂₄ =V, I; X ₂₅ =F, L, V; X ₂₆ =D or absent; X ₂₇ =T, V; X ₂₈ =F, V; and X ₂₉ =A, G

[0054] In some embodiments, a polypeptide comprises a SIRPα D1 domain variant that comprises a sequence of:

EEEX₁QX₂IQPDKSVLVAAGETX₃TLRCTX₄TS LX₅PVGPIQWFRGAGPGRX₆LIYNQX₇X₈GX₉FPRVTTVSDX₁₀TX₁₁RNNMDFSIRIGNITPADAGTYCYX₁₂KX₁₃RKGSPDDVEX₁₄KSGAGTELSVRAKPS (SEQ ID NO: 13), wherein X₁ is L, I, or V; X₂ is V, L, or I; X₃ is A or V; X₄ is A, I, or L; X₅ is I, T, S, or F; X₆ is E, V, or L; X₇ is K or R; X₈ is E or Q; X₉ is H, P, or R; X₁₀ is L, T, or G; X₁₁ is K or R; X₁₂ is V or I; X₁₃ is F, L, or V; and X₁₄ is F or V; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRPα D1 domain

that comprises the sequence of SEQ ID NO: 1.

[0055] In some embodiments, a polypeptide comprises a SIRP α D1 domain variant that comprises the sequence of SEQ ID NOs: 13, wherein X₁ is L, I, or V. In any of the aforementioned embodiments, X₂ is V, L, or, I. In some embodiments, X₃ is A or V. In some embodiments, X₄ is A, I, or L. In some embodiments, X₅ is I, T, S, or F. In some embodiments, X₆ is E, V, or L. In some embodiments, X₇ is K or R. In some embodiments, X₈ is E or Q. In some embodiments, X₉ is H, P, or R. In some embodiments, X₁₀ is L, T, or G. In some embodiments, X₁₁ is K or R. In some embodiments, X₁₂ is V or I. In some embodiments, X₁₃ is F, L, V. In some embodiments, X₁₄ is F or V. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than six amino acid substitutions relative to the wild-type SIRP α D1 domain that comprises the sequence of SEQ ID NO: 1.

[0056] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRP α D1 domain that comprises the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRP α D1 domain that comprises the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRP α D1 domain that comprises the sequence of SEQ ID NO: 1. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1 x 10⁻⁸ M, less than 5 x 10⁻⁹ M, less than 1 x 10⁻⁹ M, less 5 x 10⁻¹⁰ M, less than 1 x 10⁻¹⁰ M or less than 1 x 10⁻¹¹ M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0057] In some embodiments, a polypeptide includes a SIRP α D1 domain variant that comprises a sequence of:
EEEX₁QX₂IQPDKSVSVAAGESX₃ILHCTX₄TS LX₅PVGPIQWFRGAGPARX₆LIYNQX₇X₈G
X₉FPRVTTVSEX₁₀TX₁₁RENMDFSISISNITPADAGTYXCX₁₂KX₁₃RKGSPDTEX₁₄KSGAGT
ELSVRAKPS (SEQ ID NO: 14), wherein X₁ is L, I, or V; X₂ is V, L, or, I; X₃ is A or V; X₄ is V, I, or L; X₅ is I, T, S, or F; X₆ is E, V, or L; X₇ is K or R; X₈ is E or Q; X₉ is H, P, or R; X₁₀ is S,

T, or G; X₁₁ is K or R; X₁₂ is V or I; X₁₃ is F, L, or V; and X₁₄ is F or V; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRP α D1 domain that comprises the sequence of SEQ ID NO: 2.

[0058] In some embodiments in this aspect of the disclosure, the polypeptide comprises the sequence of SEQ ID NO: 14, wherein X₁ is L, I, or V. In some embodiments, X₂ is V, L, or I. In some embodiments, X₃ is A or V. In some embodiments, X₄ is V, I, or L. In some embodiments, X₅ is I, T, S, or F. In some embodiments, X₆ is E, V, or L. In some embodiments, X₇ is K or R. In some embodiments, X₈ is E or Q. In some embodiments, X₉ is H, P, or R. In some embodiments, X₁₀ is S, T, or G. In some embodiments, X₁₁ is K or R. In some embodiments, X₁₂ is V or I. In some embodiments, X₁₃ is F, L, or V. In some embodiments, X₁₄ is F or V. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than six amino acid substitutions relative to the wild-type SIRP α D1 domain that comprises the sequence of SEQ ID NO: 2.

[0059] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1 x 10⁻⁸ M, less than 5 x 10⁻⁹ M, less than 1 x 10⁻⁹ M, less 5 x 10⁻¹⁰ M, less than 1 x 10⁻¹⁰ M or less than 1 x 10⁻¹¹ M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0060] In some embodiments, a polypeptide includes a SIRP α D1 domain variant having a sequence of:

EEX₁X₂QX₃IQPKX₄VX₅VAAGEX₆X₇X₈LX₉CTX₁₀TSLX₁₁PVGPIQWFRGAGPX₁₂RX₁₃LIY
NQX₁₄X₁₅GX₁₆FPRVTTVSX₁₇X₁₈TX₁₉RX₂₀NMDFX₂₁IX₂₂IX₂₃NITPADAGTYYCX₂₄KX₂₅RK

GSPDX₂₆X₂₇EX₂₈KSGAGTELSVRX₂₉KPS (SEQ ID NO: 23), wherein X₁ is E or G; X₂ is L, I, or V; X₃ is V, L, or, I; X₄ is S or F; X₅ is L or S; X₆ is S or T; X₇ is A or V; X₈ is I or T; X₉ is H or R; X₁₀ is A, V, I, or L; X₁₁ is I, T, S, or F; X₁₂ is A or G; X₁₃ is E, V, or L; X₁₄ is K or R; X₁₅ is E or Q; X₁₆ is H, P, or R; X₁₇ is D or E; X₁₈ is S, L, T, or G; X₁₉ is K or R; X₂₀ is E or D; X₂₁ is S or P; X₂₂ is S or R; X₂₃ is S or G; X₂₄ is V or I; X₂₅ is F, L, V; X₂₆ is D or absent; X₂₇ is T or V; X₂₈ is F or V; and X₂₉ is A or G; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2.

[0061] In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is L, I, or V. In any of the aforementioned embodiments, X₃ is V, L, or, I. In embodiments, X₄ is S or F. In some embodiments, X₅ is L or S. In some embodiments, X₆ is S or T. In some embodiments, X₇ is A or V. In some embodiments, X₈ is I or T. In some embodiments, X₉ is H or R. In some embodiments, X₁₀ is A, V, I, or L. In some embodiments, X₁₁ is I, T, S, or F. In some embodiments, X₁₂ is A or G. In some embodiments, X₁₃ is E, V, or L. In some embodiments, X₁₄ is K or R. In some embodiments, X₁₅ is E or Q. In some embodiments, X₁₆ is H, P, or R. In some embodiments, X₁₇ is D or E. In some embodiments, X₁₈ is S, L, T, or G. In some embodiments, X₁₉ is K or R. In some embodiments, X₂₀ is E or D. In some embodiments, X₂₁ is S or P. In some embodiments, X₂₂ is S or R. In some embodiments, X₂₃ is S or G. In some embodiments, X₂₄ is V or I. In some embodiments, X₂₅ is F, L, V. In some embodiments, X₂₆ is D or absent. In some embodiments, X₂₇ is T or V. In some embodiments, X₂₈ is F or V. In some embodiments, X₂₉ is A or G. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than six amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2.

[0062] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2. In some embodiments, a SIRPα D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1 x 10⁻⁸ M, less than 5 x 10⁻⁹ M, less than 1 x 10⁻⁹ M, less 5 x 10⁻¹⁰ M, less than 1 x

10^{-10} M or less than 1×10^{-11} M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0063] In some embodiments, a polypeptide of the disclosure including a SIRP α D1 domain variant further comprises a D2 domain having the sequence of SEQ ID NO: 24, a D3 domain having the sequence of SEQ ID NO: 25, or a D2 domain having the sequence of SEQ ID NO: 24 and a D3 domain having the sequence of SEQ ID NO: 25 of a wild-type human SIRP α as shown in *Table 3*. In some embodiments, the SIRP α D1 domain variant further comprises a fragment or variant of a D2 domain or a fragment or variant of a D3 domain. In some embodiments, the SIRP α D1 domain variant further comprises a fragment or variant of a D2 domain and a fragment or variant of a D3 domain. In some embodiments, a SIRP α D1 domain variant is joined to a D2 or D3 domain by way of a linker. In some embodiments, a SIRP α D1 domain variant is joined to a D2 and D3 domain by way of a linker.

Table 3. Amino Acid Sequences of SIRP α D2 and D3 Domains

SEQ ID NO:	Description	Amino Acid Sequence
24	SIRP α D2 domain	APVVSGPAARATPQHTVSFTCESHGFSPRDITLKWFKNGNEL SDFQTNVDPVGESVSYSIHSTAKVVLTRDVDHSQVCEVAHV TLQGDPLRGTANLSETIR
25	SIRP α D3 domain	VPPTLEVTTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENG NVSRTETASTVTENKDGTYNWMSWLLVNVSAHRDDVKLTC QVEHDGQPAVSKSHDLKVS

[0064] In some embodiments, a polypeptide of the disclosure including a SIRP α D1 domain variant is attached to an Fc domain variant in order to improve the pharmacokinetic properties of the polypeptide, *e.g.*, increase serum half-life. In some embodiments, a SIRP α D1 domain variant is attached to an Fc domain variant that is unable to dimerize. In some embodiments, Fc domain variants serve to increase the serum half-life of the polypeptides described herein. In some embodiments, a polypeptide of the disclosure including a SIRP α D1 domain variant does not

include the sequence of any one of SEQ ID NOs: 26-36 shown in *Table 4*.

Table 4.

SEQ ID NO:	AMINO ACID SEQUENCE
26	EEELQVIQPDKSVSVAAGESAILHCTITSLIPVGPIQWFRGAGPARELIYNQRE GHFPRVTTVSETTRRENMDFSISISNITPADAGTY YCVKFRKGSPDTEVKSGA GTELSVRAKPS
27	EEEVQVIQPDKSVSVAAGESAILHCTLTSIPVGPIQWFRGAGPARVLIYNQR QGHFPRVTTVSEGTTRRENMDFSISISNITPADAGTY YCIKFRKGSPDTEFKSG AGTELSVRAKPS
28	EEEVQIIQPDKSVSVAAGESVILHCTITSLTPVGPIQWFRGAGPARLLIYNQRE GPFPRVTTVSETTRRENMDFSISISNITPADAGTY YCVKLRKGSPDTEFKSGA GTELSVRAKPS
29	EEELQIIQPDKSVSVAAGESAILHCTITSLSPVGPIQWFRGAGPARVLIYNQRQ GPFPRVTTVSEGTKRENMDFSISISNITPADAGTY YCIKLRKGSPDTEFKSGA GTELSVRAKPS
30	EEEIQVIQPDKSVSVAAGESVIIHCTVTSLFPVGPIQWFRGAGPARVLIYNQR QGRFPRVTTVSEGTKRENMDFSISISNITPADAGTY YCVKVRKGSPDTEVKSG GAGTELSVRAKPS
31	EEEVQIIQPDKSVSVAAGESIILHCTVTSLFPVGPIQWFRGAGPARVLIYNQRE GRFPRVTTVSEGTTRRENMDFSISISNITPADAGTY YCIKLRKGSPDTEFKSGA GTELSVRAKPS
32	EEEVQLIQPDKSVSVAAGESAILHCTVTSLFPVGPIQWFRGAGPARVLIYNQR EGPFPRVTTVSEGTKRENMDFSISISNITPADAGTY YCIKFRKGSPDTEVKSG AGTELSVRAKPS
33	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTY YCIKFRKGSPDDVEFKS GAGTELSVRAKPS
34	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARLLIYNQRQ GPFPRVTTVSETTKRENMDFSISISNITPADAGTY YCVKFRKGSPDTEFKSGA GTELSVRAKPS
35	EEEVQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQKQ GPFPRVTITSETTRRENMDFSISISNITPADAGTY YCIKFRKGSPDTEFKSGAG TELSVRAKPS

36	EEELQHQPDKSVSVAAGESAILHCTITSLTPVGPIQWFRGAGPARVLIYNQRQ GPFPRVTTVSEGTRRENMDFSISISNITPADAGTYCYKFRKGSPDTEVKSGA GTELSVRAKPS
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[0065] In some embodiments, the polypeptides and polypeptide constructs described herein are utilized *in vitro* for binding assays, such as immune assays. For example, in some embodiments, the polypeptides and polypeptide constructs described herein are utilized in liquid phase or bound to a solid phase carrier. In some embodiments, polypeptides utilized for immunoassays are detectably labeled in various ways.

[0066] In some embodiments, polypeptides and polypeptide constructs described herein are bound to various carriers and used to detect the presence of specific antigen expressing cells. Examples of carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble.

[0067] Various different labels and methods of labeling are known. Examples of labels include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bio-luminescent compounds. Various techniques for binding labels to polypeptides disclosed herein are available.

[0068] In some embodiments, the polypeptides are coupled to low molecular weight haptens. These haptens are then specifically detected by means of a second reaction. For example, in some embodiments, the hapten biotin is used with avidin or the haptens dinitrophenol, pyridoxal, or fluorescein are detected with specific anti-hapten antibodies (*e.g.*, anti-dinitrophenol antibodies, anti-pyridoxal antibodies, and anti-fluorescein antibodies respectively).

SIRPα D1 Domain Variants with Altered Glycosylation Patterns

[0069] Disclosed herein, in some embodiments, are polypeptides comprising a signal-regulatory protein α (SIRP-α) D1 variant comprising a SIRPα D1 domain, or a fragment thereof, having an amino acid mutation at residue 80 relative to a wild-type SIRPα D1 domain (*e.g.*, a wild-type SIRPα D1 domain set forth in SEQ ID NO: 1 or 2); and at least one additional amino acid mutation relative to a wild-type SIRPα D1 domain (*e.g.*, a wild-type SIRPα D1 domain set forth in SEQ ID NO: 1 or 2) at a residue selected from the group consisting of: residue 6, residue 27, residue 31, residue 47, residue 53, residue 54, residue 56, residue 66, and residue 92.

[0070] Also disclosed herein, in some embodiments, are polypeptides comprising an Fc domain variant, wherein an Fc domain variant dimer comprises two Fc domain variants, wherein each Fc domain variant independently is selected from (i) a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A; (ii) a human IgG2 Fc region consisting of mutations A330S, P331S and N297A; or (iii) a human IgG4 Fc region comprising mutations S228P, E233P, F234V, L235A, delG236, and N297A.

[0071] In some embodiments, a polypeptide in a composition disclosed herein comprises a SIRP α D1 domain variant that has reduced or minimal glycosylation. The D1 domain of SEQ ID NOs: 1 and 2 in **Table 1** each contains a single potential N-linked glycosylation site at amino acid N80 in the sequence N80ITP. Expression of a SIRP α D1 domain in Chinese Hamster Ovary (CHO) cells results in a major band of 16 kDa (non-glycosylated) and a minor band of higher molecular weight that was removed by Endo Hf. Endo Hf is a recombinant protein fusion of Endoglycosidase H and maltose binding protein. Endo Hf cleaves within the chitobiose core of high mannose and some hybrid oligosaccharides from N-linked glycoproteins. This implies that a proline at amino acid position 83 can reduce the efficiency of glycosylation, leading to a protein with different degrees of glycosylation and therefore heterogeneity. For drug development, heterogeneity can give rise to challenges in process development. Therefore, to investigate the possibility of generating homogenous, non-glycosylated forms of SIRP α D1 domain variants, in some embodiments, amino acid N80 of a SIRP α D1 variant is mutated to Ala. In some embodiments, to make a non-glycosylated, SIRP α D1 domain variant, amino acid N80 in a SIRP α D1 domain variant is replaced by any amino acid, including any naturally and non-naturally occurring amino acid, *e.g.*, N80A and N80Q. In some embodiments, a SIRP α D1 domain variant comprises an N80A mutation and at least 1 additional mutation (*e.g.*, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 additional mutations or more). In some embodiments, the additional mutation is in the CD47 binding site. In some embodiments, the additional mutation is in the hydrophobic core of the D1 domain.

[0072] In some embodiments, a polypeptide in a composition disclosed herein includes a SIRP α D1 domain variant that has increased glycosylation relative to a wild-type SIRP α D1 domain. Another option to increase homogeneity of the final product is to enhance the efficiency of glycosylation at amino acid N80 and generate SIRP α D1 domain variants with increased glycosylation relative to a wild-type. In some embodiments, the amino acid P83 in the sequence

N1TP83 affects the degree of glycosylation at amino acid N80. In some embodiments, changing P83 to any amino acid increases the efficiency of glycosylation at N80. In some embodiments, amino acid P83 in a SIRP α D1 domain variant is replaced by any amino acid, including naturally and non-naturally amino acids, *e.g.*, P83V, P83A, P83I, and P83L. In some embodiments, a polypeptide of the disclosure is expressed in a cell that is optimized not to glycosylate proteins that are expressed by such cell, for example by genetic engineering of the cell line (*e.g.*, genetically engineered yeast or mammalian host) or modifications of cell culture conditions such as addition of kifunensine or by using a naturally non-glycosylating host such as a prokaryote (*E. coli*, etc.).

[0073] **Table 5** lists specific amino acid substitutions in a SIRP α D1 domain variant relative to each D1 domain variant sequence. In some embodiments, a SIRP α D1 domain variant includes one or more (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or more) of the substitutions listed in **Table 5**. In some embodiments, the SIRP α D1 domain variants are not glycosylated or are minimally glycosylated. In some embodiments, the SIRP α D1 domain variants are fully glycosylated or almost fully glycosylated. In some embodiments, a SIRP α D1 domain variant includes at most fourteen amino acid substitutions relative to a wild-type D1 domain. In some embodiments, a SIRP α D1 domain variant includes at most ten amino acid substitutions relative to a wild-type D1 domain. In some embodiments, a SIRP α D1 domain variant includes at most seven amino acid substitutions relative to a wild-type D1 domain. In some embodiments, a SIRP α D1 domain variant of the disclosure has at least 90% (*e.g.*, at least 92%, 95%, 97% or greater than 97%) amino acid sequence identity to a sequence of a wild-type D1 domain.

[0074] In some embodiments, a SIRP α D1 domain variant is a chimeric SIRP α D1 domain variant that includes a portion of two or more wild-type D1 domains or variants thereof (*e.g.*, a portion of one wild-type D1 domain or variant thereof and a portion of another wild-type D1 domain or variant thereof). In some embodiments, a chimeric SIRP α D1 domain variant includes at least two portions (*e.g.*, three, four, five or more portions) of wild-type D1 domains or variants thereof, wherein each of the portions is from a different wild-type D1 domain. In some embodiments, a chimeric SIRP α D1 domain variant further includes one or more amino acid substitutions listed in **Table 5**.

Table 5. Amino Acid Substitutions in a SIRPa D1 Domain Variant

SEQ ID NO:	Description	Amino Acid Sequence
37	D1 domain v1	EEEX ₁ QX ₂ IQPDKSVLVAAGETX ₃ TLRCTX ₄ TS LX ₅ PVG PIQWFRGAGPGRX ₆ LIYNQX ₇ X ₈ GX ₉ FPRVTTVSDX ₁₀ T X ₁₁ RNNMDFSIRIGX ₁₂ ITX ₁₃ ADAGTYCYX ₁₄ KX ₁₅ RKGSP DDVEX ₁₆ KSGAGTELSVRKPS
-	Amino acid substitutions relative to SEQ ID NO: 37	X ₁ =L, I, V; X ₂ =V, L, I; X ₃ =A, V; X ₄ =A, I, L; X ₅ =I, T, S, F; X ₆ =E, V, L; X ₇ =K, R; X ₈ =E, Q; X ₉ =H, P, R; X ₁₀ =L, T, G; X ₁₁ =K, R; X ₁₂ =N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y; X ₁₃ =P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y; X ₁₄ =V, I; X ₁₅ =F, L, V; X ₁₆ =F, V
38	D1 domain v2	EEEX ₁ QX ₂ IQPDKSVSVAAGESX ₃ ILHCTX ₄ TS LX ₅ PVGP IQWFRGAGPARX ₆ LIYNQX ₇ X ₈ GX ₉ FPRVTTVSEX ₁₀ TX ₁ 1RENMDFSISISX ₁₂ ITX ₁₃ ADAGTYCYX ₁₄ KX ₁₅ RKGSPDT EX ₁₆ KSGAGTELSVRKPS
-	Amino acid substitutions relative to SEQ ID NO: 38	X ₁ =L, I, V; X ₂ =V, L, I; X ₃ =A, V; X ₄ =V, I, L; X ₅ =I, T, S, F; X ₆ =E, V, L; X ₇ =K, R; X ₈ =E, Q; X ₉ =H, P, R; X ₁₀ =S, T, G; X ₁₁ =K, R; X ₁₂ =N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y; X ₁₃ =P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y; X ₁₄ =V, I; X ₁₅ =F, L, V; X ₁₆ =F, V
47	Pan D1 domain	EEX ₁ X ₂ QX ₃ IQPDKX ₄ VX ₅ VAAGEX ₆ X ₇ X ₈ LX ₉ CTX ₁₀ TSL X ₁₁ PVGP IQWFRGAGPX ₁₂ RX ₁₃ LIYNQX ₁₄ X ₁₅ GX ₁₆ FPRV TTVSX ₁₇ X ₁₈ TX ₁₉ RX ₂₀ NMDFX ₂₁ IX ₂₂ IX ₂₃ X ₂₄ ITX ₂₅ ADAGT YYCX ₂₆ KX ₂₇ RKGSPDX ₂₈ X ₂₉ EX ₃₀ KSGAGTELSVRX ₃₁ KP S
-	Amino acid substitutions	X ₁ =E, G; X ₂ =L, I, V; X ₃ =V, L, I; X ₄ =S, F; X ₅ =L, S; X ₆ =S, T; X ₇ =A, V; X ₈ =I, T; X ₉ =H, R, L; X ₁₀ =A, V, I, L; X ₁₁ =I, T, S, F; X ₁₂ =A, G; X ₁₃ =E, V, L; X ₁₄ =K, R; X ₁₅ =E, Q; X ₁₆ =H,

SEQ ID NO:	Description	Amino Acid Sequence
	relative to SEQ ID NO: 47	P, R; X ₁₇ =D, E; X ₁₈ =S, L, T, G; X ₁₉ =K, R; X ₂₀ =E, N; X ₂₁ =S, P; X ₂₂ =S, R; X ₂₃ =S, G; X ₂₄ =any amino acid; X ₂₅ =any amino acid; X ₂₆ =V, I; X ₂₇ =F, L, V; X ₂₈ =D or absent; X ₂₉ =T, V; X ₃₀ =F, V; and X ₃₁ =A, G
48	Pan D1 domain	EEELQX ₁ IQPDKSVX ₂ VAAAGEX ₃ AX ₄ LX ₅ CTX ₆ TS LX ₇ PV GPIQWFRGAGPX ₈ RX ₉ LIYNQX ₁₀ X ₁₁ GX ₁₂ FPRVTTVSX ₁₃ X ₁₄ TKRX ₁₅ NMDFSIX ₁₆ IX ₁₇ X ₁₈ ITPADAGTYCYX ₁₉ KFR KGX ₂₀ X ₂₁ X ₂₂ DX ₂₃ EFGSGAGTELSVR AKPS
-	Amino acid substitutions relative to SEQ ID NO: 48	X ₁ =V, I; X ₂ =L, S; X ₃ =T, S; X ₄ =T, I; X ₅ =R, H; X ₆ =A, V, I; X ₇ =I, R, Y, K, F; X ₈ =G, A; X ₉ =E, V; X ₁₀ =K, R; X ₁₁ =E, D, Q; X ₁₂ =H, P; X ₁₃ =D, E; X ₁₄ =S, L, T; X ₁₅ =N, E; X ₁₆ =R, S; X ₁₇ =G, S; X ₁₈ =N, A; X ₁₉ =V, I; X ₂₀ =S, I, M; X ₂₁ =P or absent; X ₂₂ =D, P; and X ₂₃ =V, T
49	Pan D1 domain	EEELQX ₁ IQPDKSVLVAAAGETATLRCTX ₂ TS LX ₃ PVGPI QWFRGAGPGRX ₄ LIYNQX ₅ X ₆ GX ₇ FPRVTTVSDX ₈ TKR NNMDFSIRIGX ₉ ITPADAGTYCYX ₁₀ KFRKGSPDDVEF KSGAGTELSVR AKPS
-	Amino acid substitutions relative to SEQ ID NO: 49	X ₁ =V, I, L; X ₂ =A, I, V, L; X ₃ =I, F, S, T; X ₄ =E, V, L; X ₅ =K, R; X ₆ =E, Q; X ₇ =H, P, R; X ₈ =L, T, S, G; X ₉ =A; and X ₁₀ =V, I
50	Pan D1 domain	EEELQX ₁ IQPDKSVSVAAGESAILHCTX ₂ TS LX ₃ PVGPI QWFRGAGPARX ₄ LIYNQX ₅ X ₆ GX ₇ FPRVTTVSEX ₈ TKR ENMDFSISISX ₉ ITPADAGTYCYX ₁₀ KFRKGSPDTEFKS GAGTELSVR AKPS

SEQ ID NO:	Description	Amino Acid Sequence
-	Amino acid substitutions relative to SEQ ID NO: 50	X ₁ =V, I; X ₂ =V, I; X ₃ =I, F; X ₄ =E, V; X ₅ =K, R; X ₆ =E, Q; X ₇ =H, P; X ₈ =S, T; X ₉ =N, A; and X ₁₀ =V, I
51	Pan D1 domain	EEELQX ₁ IQPKSVLVAAGETATLRCTX ₂ TSLX ₃ PVGPI QWFRGAGPGRX ₄ LIYNQX ₅ EGX ₆ FPRVTTVSDX ₇ TKRN NMDFSIRIGX ₈ ITPADAGTYXCX ₉ KFRKGSPDDVEFKS GAGTELSVRAKPS
-	Amino acid substitutions relative to SEQ ID NO: 51	X ₁ =V, I; X ₂ =A, I; X ₃ =I, F; X ₄ =E, V; X ₅ =K, R; X ₆ =H, P; X ₇ =L, T; X ₈ =N, A; and X ₉ =V, I
52	Pan D1 domain	EEELQX ₁ IQPKSVLVAAGETATLRCTX ₂ TSLX ₃ PVGPI QWFRGAGPGRELIYNQX ₄ EGX ₅ FPRVTTVSDX ₆ TKRN NMDFSIRIGX ₇ ITPADAGTYYCVKFRKGSPDDVEFKSG AGTELSVRAKPS
-	Amino acid substitutions relative to SEQ ID NO: 52	X ₁ =V, L, I; X ₂ =A, I, L; X ₃ =I, T, S, F; X ₄ =K, R; X ₅ =H, P, R; X ₆ =L, T, G; and X ₇ =N, A
212	Pan D1 domain	EEELQX ₁ IQPKSVSVAAGESAILHCTX ₂ TSLX ₃ PVGPI QWFRGAGPARELIYNQX ₄ EGX ₅ FPRVTTVSEX ₆ TKREN MDFSISISX ₇ ITPADAGTYYCVKFRKGSPDTEFKSGAG TELSVRAKPS
-	Amino acid substitutions	X ₁ =V, L, I; X ₂ =V, I, L; X ₃ =I, T, S, F; X ₄ =K, R; X ₅ =H, P, R; X ₆ =S, T, G; and X ₇ =N, A

SEQ ID NO:	Description	Amino Acid Sequence
	relative to SEQ ID NO: 212	
218	Pan D1 domain	EEELQX ₁ IQPDKSVLVAAGETATLRCTX ₂ TSLX ₃ PVGPI QWFRGAGPGRX ₄ LIYNQX ₅ X ₆ GX ₇ FPRVTTVSDX ₈ TKR NNMDFSIRIGX ₉ X ₁₀ X ₁₁ X ₁₂ ADAGTYCYX ₁₃ KFRKGSPD DVEFKSGAGTELSVRAKPS
-	Amino acid substitutions relative to SEQ ID NO: 218	X ₁ =V, L, or I; X ₂ =A, V, L, or I; X ₃ =I, S, T, or F; X ₄ =E, L, or V; X ₅ =K or R; X ₆ =E or Q; X ₇ =H, R or P; X ₈ =S, G, L or T; X ₉ =any amino acid; X ₁₀ =any amino acid; X ₁₁ =any amino acid; X ₁₂ =any amino acid; and X ₁₃ =V or I
219	Pan D1 domain	EEELQX ₁ IQPDKSVLVAAGETATLRCTX ₂ TSLX ₃ PVGPI QWFRGAGPGRX ₄ LIYNQX ₅ X ₆ GX ₇ FPRVTTVSDX ₈ TKR NNMDFSIRIGX ₉ ITX ₁₀ ADAGTYCYX ₁₁ KFRKGSPDDVE FKSGAGTELSVRAKPS
-	Amino acid substitutions relative to SEQ ID NO: 219	X ₁ =V, L or I; X ₂ =A, V, L, or I; X ₃ =I, S, T or F; X ₄ =E, L, or V; X ₅ =K or R; X ₆ =E or Q; X ₇ =H, R or P; X ₈ =S, G, L, or T; X ₉ =N; X ₁₀ =any amino acid other than P ; and X ₁₁ =V or I

[0075] In some embodiments, a polypeptide includes a SIRPα D1 domain variant having a sequence of:

EEEX₁QX₂IQPDKSVLVAAGETX₃TLRCTX₄TSLX₅PVGPIQWFRGAGPGRX₆LIYNQX₇X₈G
X₉FPRVTTVSDX₁₀TX₁₁RNNMDFSIRIGX₁₂ITX₁₃ADAGTYCYX₁₄KX₁₅RKGSPDDVEX₁₆KS
GAGTELSVRAKPS (SEQ ID NO: 37), wherein X₁ is L, I, or V; X₂ is V, L, or I; X₃ is A or V; X₄ is A, I, or L; X₅ is I, T, S, or F; X₆ is E, V, or L; X₇ is K or R; X₈ is E or Q; X₉ is H, P, or R; X₁₀ is L, T, or G; X₁₁ is K or R; X₁₂ is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X₁₃ is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X₁₄ is V or I; X₁₅ is F, L,

or V; and X₁₆ is F or V; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1.

[0076] In some embodiments in this aspect of the disclosure, a polypeptide includes a SIRP α D1 domain variant having a sequence of SEQ ID NO: 37, wherein X₁ is L, I, or V. In some embodiments, X₂ is V, L, or, I. In some embodiments, X₃ is A or V. In some embodiments, X₄ is A, I, or L. In some embodiments, X₅ is I, T, S, or F. In some embodiments, X₆ is E, V, or L. In some embodiments, X₇ is K or R. In some embodiments, X₈ is E or Q. In some embodiments, X₉ is H, P, or R. In some embodiments, X₁₀ is L, T, or G. In some embodiments, X₁₁ is K or R. In some embodiments, X₁₂ is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y. In some embodiments, X₁₃ is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y. In some embodiments, X₁₄ is V or I. In some embodiments, X₁₅ is F, L, V. In some embodiments, X₁₆ is F or V.

[0077] In some embodiments, a polypeptide provided herein includes no more than ten amino acid substitutions relative to the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide provided herein includes no more than seven amino acid substitutions relative to the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1.

[0078] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1×10^{-8} M, less than 5×10^{-9} M, less than 1×10^{-9} M, less 5×10^{-10} M, less than 1×10^{-10} M or less than 1×10^{-11} M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0079] In some embodiments, a polypeptide includes a SIRP α D1 domain variant having a sequence of:

EEEX₁QX₂IQPDKSVSVAAGESX₃ILHCTX₄TS LX₅PVGPIQWFRGAGPARX₆LIYNQX₇X₈G
X₉FPRVTTVSEX₁₀TX₁₁RENMDFSISIX₁₂ITX₁₃ADAGTYXCX₁₄KX₁₅RKGSPDTEX₁₆KSGA
GTELSVRAKPS (SEQ ID NO: 38), wherein X₁ is L, I, or V; X₂ is V, L, or, I; X₃ is A or V; X₄
is V, I, or L; X₅ is I, T, S, or F; X₆ is E, V, or L; X₇ is K or R; X₈ is E or Q; X₉ is H, P, or R; X₁₀
is S, T, or G; X₁₁ is K or R; X₁₂ is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y;
X₁₃ is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X₁₄ is V or I; X₁₅ is F, L, or
V; and X₁₆ is F or V; and wherein the variant comprises at least one amino acid substitution
relative to a wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2.

[0080] In some embodiments in this aspect of the disclosure, a polypeptide includes a SIRP α
D1 domain variant having a sequence of SEQ ID NO: 38, wherein X₁ is L, I, or V. In some
embodiments, X₂ is V, L, or, I. In some embodiments, X₃ is A or V. In some embodiments, X₄ is
V, I, or L. In some embodiments, X₅ is I, T, S, or F. In some embodiments, X₆ is E, V, or L. In
some embodiments, X₇ is K or R. In some embodiments, X₈ is E or Q. In some embodiments, X₉
is H, P, or R. In some embodiments, X₁₀ is S, T, or G. In some embodiments, X₁₁ is K or R. In
some embodiments, X₁₂ is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y. In some
embodiments, X₁₃ is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y. In some
embodiments, X₁₄ is V or I. In some embodiments, X₁₅ is F, L, or V. In some embodiments, X₁₆
is F or V.

[0081] In some embodiments, a polypeptide includes a SIRP α D1 domain variant having no
more than ten amino acid substitutions relative to the wild-type SIRP α D1 domain having the
sequence of SEQ ID NO: 2. In some embodiments, a polypeptide includes a SIRP α D1 domain
variant having no more than seven amino acid substitutions relative to the wild-type SIRP α D1
domain having the sequence of SEQ ID NO: 2.

[0082] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater
binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In
some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity
than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some
embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than

the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1×10^{-8} M, less than 5×10^{-9} M, less than 1×10^{-9} M, less than 5×10^{-10} M, less than 1×10^{-10} M or less than 1×10^{-11} M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0083] In another aspect, the disclosure features a polypeptide including a SIRP α D1 domain variant having a sequence of:

EEX₁X₂QX₃IQPDKX₄VX₅VAAGEX₆X₇X₈LX₉CTX₁₀TSLX₁₁PVGPIQWFRGAGPX₁₂RX₁₃LIYNQX₁₄X₁₅GX₁₆FPRVTTVSX₁₇X₁₈TX₁₉RX₂₀NMDFX₂₁IX₂₂IX₂₃X₂₄ITX₂₅ADAGTYCYCX₂₆KX₂₇RKGSPDX₂₈X₂₉EX₃₀KSGAGTELSVRX₃₁KPS (SEQ ID NO: 47), wherein X₁ is E or G; X₂ is L, I, or V; X₃ is V, L, or, I; X₄ is S or F; X₅ is L or S; X₆ is S or T; X₇ is A or V; X₈ is I or T; X₉ is H, R, or L; X₁₀ is A, V, I, or L; X₁₁ is I, T, S, or F; X₁₂ is A or G; X₁₃ is E, V, or L; X₁₄ is K or R; X₁₅ is E or Q; X₁₆ is H, P, or R; X₁₇ is D or E; X₁₈ is S, L, T, or G; X₁₉ is K or R; X₂₀ is E or N; X₂₁ is S or P; X₂₂ is S or R; X₂₃ is S or G; X₂₄ is any amino acid; X₂₅ is any amino acid; X₂₆ is V or I; X₂₇ is F, L, V; X₂₈ is D or absent; X₂₉ is T or V; X₃₀ is F or V; and X₃₁ is A or G; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1 or 2.

[0084] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 47, wherein X₁ is E or G. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is L, I, or V. In any of the aforementioned embodiments, X₃ is V, L, or, I. In any of the aforementioned embodiments, X₄ is S or F. In any of the aforementioned embodiments, X₅ is L or S. In any of the aforementioned embodiments, X₆ is S or T. In any of the aforementioned embodiments, X₇ is A or V. In any of the aforementioned embodiments, X₈ is I or T. In any of the aforementioned embodiments, X₉ is H or R. In any of the aforementioned embodiments, X₁₀ is A, V, I, or L. In any of the aforementioned embodiments, X₁₁ is I, T, S, or F. In any of the aforementioned embodiments, X₁₂ is A or G. In any of the aforementioned embodiments, X₁₃ is E, V, or L. In any of the aforementioned embodiments, X₁₄ is K or R. In any of the aforementioned embodiments, X₁₅ is E or Q. In any of the aforementioned embodiments, X₁₆ is

H, P, or R. In any of the aforementioned embodiments, X₁₇ is D or E. In any of the aforementioned embodiments, X₁₈ is S, L, T, or G. In any of the aforementioned embodiments, X₁₉ is K or R. In any of the aforementioned embodiments, X₂₀ is E or N. In any of the aforementioned embodiments, X₂₁ is S or P. In any of the aforementioned embodiments, X₂₂ is S or R. In any of the aforementioned embodiments, X₂₃ is S or G. In any of the aforementioned embodiments, X₂₄ is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y. In any of the aforementioned embodiments, X₂₅ is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y. In any of the aforementioned embodiments, X₂₆ is V or I. In any of the aforementioned embodiments, X₂₇ is F, L, V. In any of the aforementioned embodiments, X₂₈ is D or absent. In any of the aforementioned embodiments, X₂₉ is T or V. In any of the aforementioned embodiments, X₃₀ is F or V. In any of the aforementioned embodiments, X₃₁ is A or G.

[0085] In some embodiments, the polypeptide of this aspect of the disclosure includes no more than ten amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than seven amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2.

[0086] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1 or 2. In some embodiments, a SIRPα D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1 × 10⁻⁸ M, less than 5 × 10⁻⁹ M, less than 1 × 10⁻⁹ M, less 5 × 10⁻¹⁰ M, less than 1 × 10⁻¹⁰ M or less than 1 × 10⁻¹¹ M. In some embodiments, a SIRPα D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0087] In some embodiments, a polypeptide includes a SIRPα D1 domain variant having a

sequence of:

EEELQX₁IQPDKSVX₂VAAGEX₃AX₄LX₅CTX₆TSX₇PVGPIQWFRGAGPX₈RX₉LIYNQX₁₀
X₁₁GX₁₂FPRVTTVSX₁₃X₁₄TKRX₁₅NMDFSIX₁₆IX₁₇X₁₈ITPADAGTYCYX₁₉KFRKGX₂₀X₂₁X₂₂
DX₂₃EFKSGAGTELSVRAPKS (SEQ ID NO: 48), wherein X₁ is V or I; X₂ is L or S; X₃ is T or S; X₄ is T or I; X₅ is R or H; X₆ is A, V, or I; X₇ is I, R, Y, K or F; X₈ is G or A; X₉ is E or V; X₁₀ is K or R; X₁₁ is E, D or Q; X₁₂ is H or P; X₁₃ is D or E; X₁₄ is S, L or T; X₁₅ is N or E; X₁₆ is R or S; X₁₇ is G or S; X₁₈ is N or A; X₁₉ is V or I; X₂₀ is S, I or M; X₂₁ is P or absent; X₂₂ is D or P; and X₂₃ is V or T, or a fragment thereof.

[0088] In another aspect, the disclosure features a polypeptide including a SIRPα D1 domain variant having a sequence of:

EEELQX₁IQPDKSVLVAAGETATLRCTX₂TSX₃PVGPIQWFRGAGPGRX₄LIYNQX₅X₆GX₇
FPRVTTVSDX₈TKRNNMDFSIRIGX₉ITPADAGTYCYX₁₀KFRKGSPDDVEFKSGAGTELSV
RAKPS (SEQ ID NO: 49), wherein X₁ is V, L, or I; X₂ is A, I, V, or L; X₃ is I, F, S, or T; X₄ is E, V, or L; X₅ is K or R; X₆ is E or Q; X₇ is H, P, or R; X₈ is L, T, S, or G; X₉ is A; and X₁₀ is V or I; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1.

[0089] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 49, wherein X₁ is V, L or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is A, I, V, or L. In any of the aforementioned embodiments, X₃ is I, F, S, or T. In any of the aforementioned embodiments, X₄ is E, V, or L. In any of the aforementioned embodiments, X₅ is K or R. In any of the aforementioned embodiments, X₆ is E or Q. In any of the aforementioned embodiments, X₇ is H, P, or R. In any of the aforementioned embodiments, X₈ is L, T, S or G. In any of the aforementioned embodiments, X₉ is A. In any of the aforementioned embodiments, X₁₀ is V or I.

[0090] In some embodiments, the polypeptide comprises a SIRPα D1 domain that comprises at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to SEQ ID NO: 49, wherein each of X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, and X₁₀ are not a wild-type amino acid.

[0091] In some embodiments, the polypeptide of this aspect of the disclosure includes no more than ten amino acid substitutions relative to the wild-type SIRPα D1 domain having the

sequence of any one of SEQ ID NO: 1. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than seven amino acid substitutions relative to the wild-type SIRP α D1 domain having the sequence of any one of SEQ ID NO: 1.

[0092] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1×10^{-8} M, less than 5×10^{-9} M, less than 1×10^{-9} M, less 5×10^{-10} M, less than 1×10^{-10} M or less than 1×10^{-11} M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0093] In another aspect, the disclosure features a polypeptide including a SIRP α D1 domain variant having a sequence of:

EEELQX₁IQPDKSVSVAAGESAILHCTX₂TSLX₃PVGPIQWFRGAGPARX₄LIYNQX₅X₆GX₇FPRVTTVSEX₈TKRENMDFSISX₉ITPADAGTYXCX₁₀KFRKGGSPDTEFKSGAGTELSVR AKPS, (SEQ ID NO: 50), wherein X₁ is V or I; X₂ is V or I; X₃ is I or F; X₄ is E or V; X₅ is K or R; X₆ is E or Q; X₇ is H or P; X₈ is S or T; X₉ is N or A; and X₁₀ V or I; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2.

[0094] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 50, wherein X₁ is V or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is V or I. In any of the aforementioned embodiments, X₃ is I or F. In any of the aforementioned embodiments, X₄ is E or V. In any of the aforementioned embodiments, X₅ is K or R. In any of the aforementioned embodiments, X₆ is E or Q. In any of the aforementioned embodiments, X₇ is H or P. In any of the aforementioned embodiments, X₈ is S or R. In any of

the aforementioned embodiments, X₉ is N or A. In any of the aforementioned embodiments, X₁₀ is V or L.

[0095] In some embodiments, the polypeptide comprises a SIRPα D1 domain that comprises at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to SEQ ID NO: 50, wherein each of X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, and X₁₀ is not a wild-type amino acid.

[0096] In some embodiments, the polypeptide of this aspect of the disclosure includes no more than ten amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than seven amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 2.

[0097] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, a SIRPα D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1 × 10⁻⁸ M, less than 5 × 10⁻⁹ M, less than 1 × 10⁻⁹ M, less 5 × 10⁻¹⁰ M, less than 1 × 10⁻¹⁰ M or less than 1 × 10⁻¹¹ M. In some embodiments, a SIRPα D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0098] In another aspect, the disclosure features a polypeptide including a SIRPα D1 domain variant having a sequence of:

EEELQX₁IQPDKSVLVAAGETATLRCTX₂TSLX₃PVGPIQWFRGAGPGRX₄LIYNQX₅EGX₆
FPRVTTVSDX₇TKRNNMDFSIRIGX₈ITPADAGTYXCX₉KFRKGSPDDVEFKSGAGTELSV
RAKPS (SEQ ID NO: 51), wherein X₁ is V or I; X₂ is A or I; X₃ is I or F; X₄ is E or V; X₅ is K or R; X₆ is H or P; X₇ is L or T; X₈ is N or A; and X₉ is V or I; and wherein the variant comprises at

least one amino acid substitution relative to a wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1.

[0099] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 51, wherein X₁ is V or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is A or I. In any of the aforementioned embodiments, X₃ is I or F. In any of the aforementioned embodiments, X₄ is E or V. In any of the aforementioned embodiments, X₅ is K or R. In any of the aforementioned embodiments, X₆ is H or P. In any of the aforementioned embodiments, X₇ is L or T. In any of the aforementioned embodiments, X₈ is N or A. In any of the aforementioned embodiments, X₉ is V or I. In some embodiments, X₄ is not V.

[0100] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 51, wherein X₈ is A. In any of the aforementioned embodiments in this aspect of the disclosure, X₈ is A and X₁ is V or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₈ is A and X₂ is A or I. In any of the aforementioned embodiments, X₈ is A and X₃ is I or F. In any of the aforementioned embodiments, X₈ is A and X₄ is E or V. In some embodiments, X₄ is not V. In any of the aforementioned embodiments, X₈ is A and X₅ is K or R. In any of the aforementioned embodiments, X₈ is A and X₆ is H or P. In any of the aforementioned embodiments, X₈ is A and X₇ is A or V. In any of the aforementioned embodiments, X₈ is A and X₉ is V or I.

[0101] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 51, wherein X₈ is A. In any of the aforementioned embodiments in this aspect of the disclosure, X₈ is A and X₁ is I. In any of the aforementioned embodiments in this aspect of the disclosure, X₈ is A and X₂ is I. In any of the aforementioned embodiments, X₈ is A and X₃ is F. In any of the aforementioned embodiments, X₈ is A and X₄ is V. In any of the aforementioned embodiments, X₈ is A and X₅ is R. In any of the aforementioned embodiments, X₈ is A and X₆ is P. In any of the aforementioned embodiments, X₈ is A and X₇ is T. In any of the aforementioned embodiments, X₈ is A and X₉ is I.

[0102] In some embodiments, the polypeptide comprises a SIRP α D1 domain variant that comprises at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to SEQ ID NO: 51, wherein each of X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, and X₉ is not a wild-type amino acid.

[0103] In some embodiments, the polypeptide of this aspect of the disclosure comprises no more than ten amino acid substitutions relative to the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide of this aspect of the disclosure comprises no more than seven amino acid substitutions relative to the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1.

[0104] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NOs: 1. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1×10^{-8} M, less than 5×10^{-9} M, less than 1×10^{-9} M, less 5×10^{-10} M, less than 1×10^{-10} M or less than 1×10^{-11} M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0105] In another aspect, the disclosure features a polypeptide including a SIRP α D1 domain variant having a sequence of:

EEELQX₁IQPDKSVLVAAGETATLRCTX₂TSLX₃PVGPIQWFRGAGPGRELIYNQX₄EGX₅F
PRVTTVSDX₆TKRNNMDFSIRIGX₇ITPADAGTYCYVKFRKGSPDDVEFKSGAGTELSVR
AKPS (SEQ ID NO: 222), wherein X₁ is V, L, or I; X₂ is A, I, or L; X₃ is I, T, S, or F; X₄ is K or R; X₅ is H or P; X₆ is L, T, or G; X₇ is N or A; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRP α D1 domain having a sequence according to SEQ ID NO: 1.

[0106] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 222, wherein X₁ is V, L, or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is A, I, or L. In any of the aforementioned embodiments, X₃ is I, T, S, or F. In any of the aforementioned embodiments, X₄ is K or R. In any of the aforementioned embodiments,

X₅ is H or P. In any of the aforementioned embodiments, X₆ is L, T, or G. In any of the aforementioned embodiments, X₇ is N or A.

[0107] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 222, wherein X₁ is V or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is A or I. In any of the aforementioned embodiments, X₃ is I or F. In any of the aforementioned embodiments, X₄ is K or R. In any of the aforementioned embodiments, X₅ is H or P. In any of the aforementioned embodiments, X₆ is L or T. In any of the aforementioned embodiments, X₇ is N or A.

[0108] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 222, wherein X₇ is A. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₁ is V or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₂ is A or I. In any of the aforementioned embodiments, X₇ is A and X₃ is I or F. In any of the aforementioned embodiments, X₇ is A and X₄ is K or R. In any of the aforementioned embodiments, X₇ is A and X₅ is H or P. In any of the aforementioned embodiments, X₇ is A and X₆ is L or T.

[0109] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 222, wherein X₇ is A. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₁ is I. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₂ is I. In any of the aforementioned embodiments, X₇ is A and X₃ is F. In any of the aforementioned embodiments, X₇ is A and X₄ is R. In any of the aforementioned embodiments, X₇ is A and X₅ is P. In any of the aforementioned embodiments, X₇ is A and X₆ is T.

[0110] In some embodiments, the polypeptide comprises a SIRPα D1 domain that comprises at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to SEQ ID NO: 222, wherein each of X₁, X₂, X₃, X₄, X₅, X₆, and X₇ is not a wild-type amino acid.

[0111] In some embodiments, the polypeptide of this aspect of the disclosure includes no more than ten amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than seven amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 1.

[0112] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 1. In some embodiments, fragments include polypeptides of less than 10 amino acids in length, about 10 amino acids in length, about 20 amino acids in length, about 30 amino acids in length, about 40 amino acids in length, about 50 amino acids in length, about 60 amino acids in length, about 70 amino acids in length, about 80 amino acids in length, about 90 amino acids in length, about 100 amino acids in length, or more than about 100 amino acids in length. Fragments retain the ability to bind to CD47. Preferably, SIRP α D1 domain variant polypeptides and fragments thereof bind to CD47 with a higher affinity than a SIRP α polypeptide binds to CD47. For example, in some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1×10^{-8} M, less than 5×10^{-9} M, less than 1×10^{-9} M, less 5×10^{-10} M, less than 1×10^{-10} M or less than 1×10^{-11} M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0113] In another aspect, the disclosure features a polypeptide including a SIRP α D1 domain variant having a sequence of:
 EEELQX₁IQPDKSVSVAAGESAILHCTX₂TSLX₃PVGPIQWFRGAGPARELIYNQX₄EGX₅FP
 RVTTVSEX₆TKRENMDFSISIX₇ITPADAGTYCYVKFRKGSPDTEFKSGAGTELSVRAKP
 S (SEQ ID NO: 212), wherein X₁ is V, L, or I; X₂ is V, I, or L; X₃ is I, T, S, or F; X₄ is K or R; X₅ is H, P, or R; X₆ is S, T, or G; X₇ is N or A; and wherein the variant comprises at least one amino acid substitution relative to a wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2.

[0114] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 212, wherein X₁ is V, L, or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is V, I, or L. In any of the aforementioned embodiments, X₃ is I, T, S, or F. In any

of the aforementioned embodiments, X₄ is K or R. In any of the aforementioned embodiments, X₅ is H or P. In any of the aforementioned embodiments, X₆ is S, T, or G. In any of the aforementioned embodiments, X₇ is N or A.

[0115] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 212, wherein X₁ is V or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₂ is V or I. In any of the aforementioned embodiments, X₃ is I or F. In any of the aforementioned embodiments, X₄ is K or R. In any of the aforementioned embodiments, X₅ is H or P. In any of the aforementioned embodiments, X₆ is S or T. In any of the aforementioned embodiments, X₇ is N or A.

[0116] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 212, wherein X₇ is A. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₁ is V or I. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₂ is V or I. In any of the aforementioned embodiments, X₇ is A and X₃ is I or F. In any of the aforementioned embodiments, X₇ is A and X₄ is K or R. In any of the aforementioned embodiments, X₇ is A and X₅ is H or P. In any of the aforementioned embodiments, X₇ is A and X₆ is S or T.

[0117] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 212, wherein X₇ is A. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₁ is I. In any of the aforementioned embodiments in this aspect of the disclosure, X₇ is A and X₂ is I. In any of the aforementioned embodiments, X₇ is A and X₃ is F. In any of the aforementioned embodiments, X₇ is A and X₄ is R. In any of the aforementioned embodiments, X₇ is A and X₅ is P. In any of the aforementioned embodiments, X₇ is A and X₆ is T.

[0118] In some embodiments, the polypeptide comprises a SIRPα D1 domain having at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to SEQ ID NO: 212, wherein each of X₁, X₂, X₃, X₄, X₅, X₆, and X₇ is not a wild-type amino acid.

[0119] In some embodiments, the polypeptide of this aspect of the disclosure includes no more than ten amino acid substitutions relative to the wild-type SIRPα D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide of this aspect of the disclosure includes no more than seven amino acid substitutions relative to the wild-type SIRPα

D1 domain having the sequence of SEQ ID NO: 2.

[0120] In some embodiments, the polypeptide binds CD47 with at least 10-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide binds CD47 with at least 100-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, the polypeptide binds CD47 with at least 1000-fold greater binding affinity than the wild-type SIRP α D1 domain having the sequence of SEQ ID NO: 2. In some embodiments, fragments include polypeptides of less than 10 amino acids in length, about 10 amino acids in length, about 20 amino acids in length, about 30 amino acids in length, about 40 amino acids in length, about 50 amino acids in length, about 60 amino acids in length, about 70 amino acids in length, about 80 amino acids in length, about 90 amino acids in length, about 100 amino acids in length, or more than about 100 amino acids in length. Fragments retain the ability to bind to CD47. Preferably, SIRP α D1 domain variant polypeptides and fragments thereof bind to CD47 with a higher affinity than a SIRP α polypeptide binds to CD47. For example, in some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D less than 1×10^{-8} M, less than 5×10^{-9} M, less than 1×10^{-9} M, less 5×10^{-10} M, less than 1×10^{-10} M or less than 1×10^{-11} M. In some embodiments, a SIRP α D1 domain variant polypeptide or fragment thereof binds to CD47 with a K_D between about 500 nM and 100 nM, between about 100 nM and 50 nM, between about 50 nM and 10 nM, between about 10 nM and 5 nM, between about 5 nM and 1 nM, between about 1 nM and 500 pM, between about 500 pM and 100 pM, between about 100 pM and 50 pM, or between about 50 pM and 10 pM.

[0121] Described herein, in some embodiments, is a polypeptide comprising a SIRP α D1 domain variant having a sequence according to:
 EEELQX₁IQPKSVLVAAGETATLRCTX₂TSLX₃PVGPIQWFRGAGPGRX₄LIYNQX₅X₆GX₇
 FPRVTTVSDX₈TKRNNMDFSIRIGX₉X₁₀X₁₁X₁₂ADAGTYCYX₁₃KFRKGSPDDVEFKSGAG
 TELSVRAKPS (SEQ ID NO: 218), wherein X₁ is V, L, or I; X₂ is A, V, L, or I; X₃ is I, S, T, or F; X₄ is E, L, or V; X₅ is K or R; X₆ is E or Q; X₇ is H, R, or P; X₈ is S, G, L, or T; X₉ is any amino acid; X₁₀ is any amino acid; X₁₁ is any amino acid; X₁₂ is any amino acid; and X₁₃ is V or I; and wherein the SIRP α D1 domain variant comprises at least two amino acid substitutions relative to a wild-type SIRP α D1 domain having a sequence according to SEQ ID NO: 1.

[0122] In some embodiments, the polypeptide comprises the sequence of SEQ ID NO: 212, wherein X₁, wherein X₉ is A. In any of the aforementioned embodiments in this aspect of the disclosure, X₉ is N. In any of the aforementioned embodiments in this aspect of the disclosure X₁₀ is I. In any of the aforementioned embodiments in this aspect of the disclosure X₉ is N and X₁₀ is P. In any of the aforementioned embodiments in this aspect of the disclosure X₉ is N and X₁₁ is any amino acid other than S, T, or C. In any of the aforementioned embodiments in this aspect of the disclosure X₁₁ is T. In any of the aforementioned embodiments in this aspect of the disclosure X₁₁ is an amino acid other than T. In any of the aforementioned embodiments in this aspect of the disclosure X₁₂ is P. In any of the aforementioned embodiments in this aspect of the disclosure X₉ is N and X₁₂ is any amino acid other than P.

[0123] Described herein, in some embodiments, is a polypeptide comprising a SIRPα D1 domain variant having a sequence according to:

EEELQX₁IQPKSVLVAAGETATLRCTX₂TSX₃PVGPIQWFRGAGPGRX₄LIYNQX₅X₆GX₇FPRVTTVSDX₈TKRNNMDF SIRIGX₉ITX₁₀ADAGTYXCX₁₁KFRKGGSPDDVEFKSGAGTEL SVRAKPS (SEQ ID NO: 219), wherein X₁ is V, L, or I; X₂ is A, V, L, or I; X₃ is I, S, T, or F; X₄ is E, L, or V; X₅ is K or R; X₆ is E or Q; X₇ is H, R, or P; X₈ is S, G, L, or T; X₉ is N; X₁₀ is any amino acid other than P; and X₁₁ is V or I; and wherein the SIRPα D1 domain variant comprises at least two amino acid substitutions relative to a wild-type SIRPα D1 domain having a sequence according to SEQ ID NO: 1.

[0124] In another aspect of the disclosure, compositions are disclosed herein which include a SIRPα D1 domain variant polypeptide having the amino acid sequence of SEQ ID NO: 48, or a fragment thereof. In some embodiments, the SIRPα D1 domain variant polypeptide or fragment thereof binds to CD47 with a higher affinity compared to the affinity that a SIRPα polypeptide binds to the CD47. In some embodiments, the SIRPα D1 domain variant polypeptide binds to CD47 with a K_D less than 1 × 10⁻⁸M, or less than 1 × 10⁻⁹M, less than 1 × 10⁻¹⁰M or less than 1 × 10⁻¹¹M. In some embodiments, the above-mentioned SIRPα D1 domain variant polypeptides are attached or fused to a second polypeptide. In some embodiments, the second polypeptide includes, without limitation, an Fc polypeptide, an Fc variant or a fragment of the foregoing.

[0125] Without limiting the foregoing, in some embodiments, a SIRPα D1 domain variant polypeptide is selected from any one of SEQ ID NOs: 53-87 and 213 shown in *Table 6*.

Table 6. *SIRPa Variant Polypeptides*

SEQ ID NO:	Amino Acid Sequence
53	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQRQ GPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGAG TELSVRAKPS
54	EEELQVIQPDKSVSVAAGESAILHCTVTSLFPVGPIQWFRGAGPARELIYNQR QGPFPRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSG AGTELSVRAKPS
55	EEELQVIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQR QGPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGA GTELSVRAKPS
56	EEELQIIQPDKSVSVAAGESAILHCTVTSLFPVGPIQWFRGAGPARVLIYNQR QGPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGA GTELSVRAKPS
57	EEELQIIQPDKSVSVAAGESAILHCTITSLIPVGPIQWFRGAGPARVLIYNQRQ GPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGAG TELSVRAKPS
58	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARELIYNQRQ GPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGAG TELSVRAKPS
59	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQKQ GPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGAG TELSVRAKPS
60	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQRE GPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGAG TELSVRAKPS

SEQ ID NO:	Amino Acid Sequence
61	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQRQ GHFPRVTTVSETTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGA GTELSVRAKPS
62	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQRQ GPFPRVTTVSESTKRENMDFSISISNITPADAGTYYCIKFRKGSPDTEFKSGAG TELSVRAKPS
63	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQRQ GPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGA GTELSVRAKPS
64	EEELQVIQPDKSVSVAAGESAILHCTVTSLIPVGPIQWFRGAGPARELIYNQR EGPFPRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSG AGTELSVRAKPS
65	EEELQVIQPDKSVSVAAGESAILHCTVTSLFPVGPIQWFRGAGPARELIYNQR EGPFPRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSG AGTELSVRAKPS
66	EEELQVIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARELIYNQR EGPFPRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSG AGTELSVRAKPS
67	EEELQVIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARELIYNQR EGPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSG AGTELSVRAKPS
68	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARELIYNQRE GPFPRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGA GTELSVRAKPS

SEQ ID NO:	Amino Acid Sequence
69	EEELQVIQPDKSVSVAAGESAILHCTITSLIPVGPIQWFRGAGPARELIYNQRE GPFPRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGA GTELSVRAKPS
70	EEELQIIQPDKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARELIYNQRE GPFPRVTTVSETTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGA GTELSVRAKPS
71	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEF KSGAGTELSVRAKPS
72	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKS GAGTELSVRAKPS
73	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFK SGAGTELSVRAKPS
74	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKS GAGTELSVRAKPS
75	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFK SGAGTELSVRAKPS
76	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ REGPFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEF KSGAGTELSVRAKPS

SEQ ID NO:	Amino Acid Sequence
77	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRTTVSDTTKRNNMDFSIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPS
78	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPS
79	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRTTVSDLTNRNNMDFSIRIGAITPADAGTYCYCVKFRKGSPDDVEF KSGAGTELSVRAKPS
80	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR EGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPS
81	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ REGPFPRTTVSDLTNRNNMDFSIRIGAITPADAGTYCYCVKFRKGSPDDVEF KSGAGTELSVRAKPS
82	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRTTVSDLTNRNNMDFSIRIGAITPADAGTYCYCVKFRKGSPDDVEFK SGAGTELSVRAKPS
83	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDLTNRNNMDFSIRIGAITPADAGTYCYCVKFRKGSPDDVEFKS GAGTELSVRAKPS
84	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYCYCVKFRKGSPDDVEFK SGAGTELSVRAKPS

SEQ ID NO:	Amino Acid Sequence
85	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFKS GAGTELSVRAKPS
86	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTY YCIKFRKGSPDDVEFKS GAGTELSVRAKPS
87	EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQK EGHFPRVTTVSDLTNRNNMDFSIRIGNITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPS
195	EEELQIIQPDKSVLVAAGETATLRCTMTSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPS
196	EEELQIIQPDKSVLVAAGETATLRCTITSLKPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPS
197	EEELQIIQPDKSVLVAAGETATLRCTITSLRPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPS
198	EEELQIIQPDKSVLVAAGETATLRCTITSLYPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPS
199	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR DGPFRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPS

SEQ ID NO:	Amino Acid Sequence
200	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGIPDDVEFKSG AGTELSVRAKPS
201	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGMPDDVEFKS GAGTELSVRAKPS
202	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGGSPDVEFKSG AGTELSVRAKPS
203	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGSSEPDVEFKS GAGTELSVRAKPS
204	EEELQIIQPDKSVLVAAGETATLRCTITSLRPVGPIQWFRGAGPGRELIYNQR DGPFPVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGGSPDDVEFK SGAGTELSVRAKPS
205	EEELQIIQPDKSVLVAAGETATLRCTITSLRPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGIPDDVEFKS GAGTELSVRAKPS
206	EEELQIIQPDKSVLVAAGETATLRCTITSLRPVGPIQWFRGAGPGRELIYNQR DGPFPVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGIPDDVEFKS GAGTELSVRAKPS
207	EEELQIIQPDKSVLVAAGETATLRCTITSLYPVGPIQWFRGAGPGRELIYNQR DGPFPVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGGSPDDVEFK SGAGTELSVRAKPS

SEQ ID NO:	Amino Acid Sequence
208	EEELQIIQPDKSVLVAAGETATLRCTITSLYPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGIPDDVEFKS GAGTELSVRAKPS
209	EEELQIIQPDKSVLVAAGETATLRCTITSLYPVGPIQWFRGAGPGRELIYNQR DGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGIPDDVEFKS GAGTELSVRAKPS
210	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR DGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGIPDDVEFKS GAGTELSVRAKPS
213	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRTTVSDLTNRNNMDFSIRIGNITVADAGTYYCVKFRKGSPDDVEF KSGAGTELSVRAKPS

[0126] In some embodiments, the polypeptide comprises a SIRP α D1 domain variant that has at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to any variant provided in **Table 6**.

[0127] In some embodiments, the polypeptide comprises a SIRP α D1 domain that has at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to SEQ ID NOs: 80, 81, or 85 in **Table 6**.

Fc Domain Variants and Fusion Polypeptides Comprising Same

[0128] Disclosed herein, in some embodiments, are polypeptides comprising a signal-regulatory protein α (SIRP- α) D1 variant comprising a SIRP α D1 domain, or a fragment thereof, having an amino acid mutation at residue 80 relative to a wild-type SIRP α D1 domain (*e.g.*, a wild-type SIRP α D1 domain set forth in SEQ ID NO: 1 or 2); and at least one additional amino acid mutation relative to a wild-type SIRP α D1 domain (*e.g.*, a wild-type SIRP α D1 domain set

forth in SEQ ID NO: 1 or 2) at a residue selected from the group consisting of: residue 6, residue 27, residue 31, residue 47, residue 53, residue 54, residue 56, residue 66, and residue 92.

[0129] Also disclosed herein, in some embodiments, are Fc domain variant dimers, wherein the Fc domain variant dimer comprises two Fc domain variants, wherein each Fc domain variant independently is selected from (i) a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A; (ii) a human IgG2 Fc region consisting of mutations A330S, P331S and N297A; or (iii) a human IgG4 Fc region comprising mutations S228P, E233P, F234V, L235A, delG236, and N297A.

[0130] Antibodies that target cell surface antigens can trigger immunostimulatory and effector functions that are associated with Fc receptor (FcR) engagement on immune cells. There are a number of Fc receptors that are specific for particular classes of antibodies, including IgG (gamma receptors), IgE (eta receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of the Fc region to Fc receptors on cell surfaces can trigger a number of biological responses including phagocytosis of antibody-coated particles (antibody-dependent cell-mediated phagocytosis, or ADCP), clearance of immune complexes, lysis of antibody-coated cells by killer cells (antibody-dependent cell-mediated cytotoxicity, or ADCC) and, release of inflammatory mediators, placental transfer, and control of immunoglobulin production. Additionally, binding of the C1 component of complement to antibodies can activate the complement system. Activation of complement can be important for the lysis of cellular pathogens. However, the activation of complement can also stimulate the inflammatory response and can also be involved in autoimmune hypersensitivity or other immunological disorders. Variant Fc regions with reduced or ablated ability to bind certain Fc receptors are useful for developing therapeutic antibodies and Fc-fusion polypeptide constructs which act by targeting, activating, or neutralizing ligand functions while not damaging or destroying local cells or tissues.

[0131] In some embodiments, a SIRP α D1 polypeptide construct comprises a non-naturally occurring SIRP α D1 domain variant linked to an Fc domain variant which forms an Fc domain having ablated or reduced effector function.

[0132] In some embodiments, a Fc domain variant refers to a polypeptide chain that includes second and third antibody constant domains (e.g., CH2 and CH3). In some embodiments, an Fc

domain variant also includes a hinge domain. In some embodiments, the Fc domain variant is of any immunoglobulin antibody isotype, including IgG, IgE, IgM, IgA, and IgD. Additionally, in some embodiments, an Fc domain variant is of any IgG subtype (*e.g.*, IgG1, IgG2, IgG2a, IgG2b, IgG2c, IgG3, and IgG4). In some embodiments, an Fc domain variant comprises as many as ten amino acid modifications (*e.g.*, insertions, deletions and/or substitutions) relative to a wild-type Fc domain monomer sequence (*e.g.*, 1-10, 1-8, 1-6, 1-4 amino acid substitutions, additions or insertions, deletions, or combinations thereof) that alter the interaction between an Fc domain and an Fc receptor.

[0133] As used herein, the term “Fc domain dimer” refers to a dimer of two Fc domains. In a wild-type Fc domain dimer, two wild-type Fc domains dimerize by the interaction between the two CH3 antibody constant domains, as well as one or more disulfide bonds that form between the hinge domains of the two dimerized Fc domains.

[0134] As used herein, the term “Fc domain dimer variant” comprises at least one Fc domain variant. In some embodiments, an Fc domain dimer variant comprises Fc domain variants that are mutated to lack effector functions, for example a “dead Fc domain dimer variant.” In some embodiments, each of the Fc domains in an Fc domain dimer variant includes amino acid substitutions in the CH2 antibody constant domain to reduce the interaction or binding between the Fc domain dimer variant and an Fc receptor, such as an Fcγ receptor (FcγR), an Fcα receptor (FcαR), or an Fcε (FcεR).

[0135] In some embodiments, a SIRPα D1 domain variant (*e.g.*, any of the variants described in Tables 2, 5, and 6) is fused to an Fc domain variant of an immunoglobulin or a fragment of an Fc domain variant. In some embodiments, an Fc domain variant of an immunoglobulin or a fragment of an Fc domain variant is capable of forming an Fc domain dimer with another Fc domain variant. In some embodiments, an Fc domain variant of an immunoglobulin or a fragment of an Fc domain variant is not capable of forming an Fc domain dimer with another Fc domain variant. In some embodiments, an Fc domain variant or a fragment of an Fc domain variant is fused to a polypeptide of the disclosure to increase serum half-life of the polypeptide. In some embodiments, an Fc domain variant or a fragment of an Fc domain variant fused to a polypeptide of the disclosure dimerizes with a second Fc domain variant to form an Fc domain dimer variant which binds an Fc receptor, or alternatively, an Fc domain variant binds to an Fc

receptor. In some embodiments, an Fc domain variant or a fragment of the Fc domain variant fused to a polypeptide to increase serum half-life of the polypeptide does not induce any immune system-related response.

[0136] In some embodiments, a SIRP α polypeptide or construct provided herein includes a SIRP α D1 domain or variant thereof joined to a first Fc domain variant and an antibody variable domain joined to a second Fc domain variant, in which the first and second Fc domain variants combine to form an Fc domain dimer variant (*e.g.*, a heterodimeric Fc domain dimer variant). An Fc domain dimer is the protein structure that is found at the C-terminus of an immunoglobulin. An Fc domain dimer includes two Fc domains that are dimerized by the interaction between the CH3 antibody constant domains. A wild-type Fc domain dimer forms the minimum structure that binds to an Fc receptor, *e.g.*, Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIIa, Fc γ RIIIb, and Fc γ RIV.

[0137] The Fc domain dimer is not involved directly in binding an antibody to its target, but can be involved in various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity. In some embodiments, the Fc domain in a SIRP α polypeptide or construct of the disclosure comprises amino acid substitutions, additions or insertions, deletions, or any combinations thereof that lead to decreased effector function such as decreased antibody-dependent cell-mediated cytotoxicity (ADCC), decreased complement-dependent cytotoxicity (CDC), decreased antibody-dependent cell-mediated phagocytosis (ADCP), or any combinations thereof. In some embodiments, the SIRP α polypeptides or constructs of the disclosure are characterized by decreased binding (*e.g.*, minimal binding or absence of binding) to a human Fc receptor and decreased binding (*e.g.*, minimal binding or absence of binding) to complement protein C1q. In some embodiments, the SIRP α constructs of the disclosure are characterized by decreased binding (*e.g.*, minimal binding or absence of binding) to human Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIIb, or any combinations thereof, and C1q. To alter or reduce an antibody-dependent effector function, such as ADCC, CDC, ADCP, or any combinations thereof, in some embodiments, the Fc domains in SIRP α constructs of the disclosure are of the IgG class and comprise one or more amino acid substitutions at E233, L234, L235, G236, G237, D265, D270, N297, E318, K320, K322, A327, A330, P331, or P329 (numbering according to the EU index of Kabat (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991))).

[0138] In some embodiments, polypeptide constructs comprising a non-native Fc region described herein exhibit reduced or ablated binding to at least one of Fcγ receptors CD16a, CD32a, CD32b, CD32c, and CD64 as compared to a polypeptide construct comprising a native Fc region. In some cases, the polypeptide constructs described herein exhibit reduced or ablated binding to CD16a, CD32a, CD32b, CD32c, and CD64 Fcγ receptors.

[0139] CDC refers to a form of cytotoxicity in which the complement cascade is activated by the complement component C1q binding to antibody Fc domains. In some embodiments, polypeptide constructs comprising a non-native Fc region described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in C1q binding compared to a polypeptide construct comprising a wild-type Fc region. In some cases, polypeptide constructs comprising a non-native Fc region as described herein exhibit reduced CDC as compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs comprising a non-native Fc region as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in CDC compared to a polypeptide construct comprising a wild-type Fc region. In some cases, polypeptide constructs comprising a non-natural Fc domain variants or Fc domain dimer variants as described herein exhibit negligible CDC as compared to a polypeptide construct comprising a wild-type Fc region.

[0140] In some embodiments, the Fc domain variants or Fc domain dimer variants described herein are minimally glycosylated or have reduced glycosylation relative to a wild-type sequence. In some embodiments, deglycosylation is accomplished with a mutation of N297A, or by mutating N297 to any amino acid which is not N. In some embodiments, deglycosylation is accomplished by disrupting the motif N-Xaa1-Xaa2-Xaa3, wherein N = asparagine; Xaa1 = any amino acid except P (proline); Xaa2 = T (threonine), S (serine) or C (cysteine); and Xaa3 = any amino acid except P (proline). In one embodiment, the N-Xaa1-Xaa2-Xaa3 motif refers to residues 297-300 as designated according to Kabat et al., 1991. In some embodiments, a mutation to any one or more of N, Xaa1, Xaa2, or Xaa3 results in deglycosylation of the Fc domain variant or Fc domain dimer variant.

[0141] In some embodiments, variants of antibody IgG constant regions (*e.g.*, Fc domain variants or Fc domain dimer variants) possess a reduced capacity to specifically bind Fcγ

receptors or have a reduced capacity to induce phagocytosis. In some embodiments, variants of antibody IgG constant regions (*e.g.*, Fc domain variants or Fc domain dimer variants) possess a reduced capacity to specifically bind Fcγ receptors and have a reduced capacity to induce phagocytosis. For example, in some embodiments, an Fc domain variant is mutated to lack effector functions, typical of a “dead” Fc domain variant. For example, in some embodiments, an Fc domain variant includes specific amino acid substitutions that are known to minimize the interaction between the Fc domain dimer and an Fcγ receptor. In some embodiments, an Fc domain variant is from an IgG1 antibody and includes one or more of amino acid substitutions L234A, L235A, G237A, and N297A (as designated according to the EU numbering system per Kabat et al., 1991). In some embodiments, one or more additional mutations are included in such IgG1 Fc domain variant. Non-limiting examples of such additional mutations for human IgG1 Fc domain variants include E318A and K322A. In some instances, a human IgG1 Fc domain variant has up to 12, 11, 10, 9, 8, 7, 6, 5 or 4 or fewer mutations in total as compared to wild-type human IgG1 sequence. In some embodiments, one or more additional deletions are included in such IgG1 Fc domain variant. For example, in some embodiments, the C-terminal lysine of the Fc domain IgG1 heavy chain constant region provided in SEQ ID NO: 88 in **Table 7** is deleted, for example to increase the homogeneity of the polypeptide when the polypeptide is produced in bacterial or mammalian cells. In some instances, a human IgG1 Fc domain variant has up to 12, 11, 10, 9, 8, 7, 6, 5 or 4 or fewer deletions in total as compared to wild-type human IgG1 sequence (see, *e.g.*, SEQ ID NO: 161 below). In some embodiments, a IgG1 Fc domain variant has a sequence according to any one of SEQ ID NO: 135, SEQ ID NO: 136 or SEQ ID NO: 137. SEQ ID NO: 161:

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DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG
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[0142] In some embodiments, an Fc domain variant is from an IgG2 or IgG4 antibody and includes amino acid substitutions A330S, P331S, or both A330S and P331S. The aforementioned amino acid positions are defined according to Kabat, et al. (1991). The Kabat numbering of amino acid residues can be determined for a given antibody by alignment at regions of homology

of the sequence of the antibody with a “standard” Kabat numbered sequence. In some embodiments, the Fc domain variant comprises a human IgG2 Fc domain sequence comprising one or more of A330S, P331S and N297A amino acid substitutions (as designated according to the EU numbering system per Kabat, et al. (1991)). In some embodiments, one or more additional mutations are included in such IgG2 Fc domain variants. Non-limiting examples of such additional mutations for human IgG2 Fc domain variant include V234A, G237A, P238S, V309L and H268A (as designated according to the EU numbering system per Kabat et al. (1991)). In some instances, a human IgG2 Fc domain variant has up to 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or fewer mutations in total as compared to wild-type human IgG2 sequence. In some embodiments, one or more additional deletions are included in such IgG2 Fc domain variant. For example, in some embodiments, the C-terminal lysine of the Fc domain IgG2 heavy chain constant region provided in SEQ ID NO: 89 in **Table 7** is deleted, for example to increase the homogeneity of the polypeptide when the polypeptide is produced in bacterial or mammalian cells. In some instances, a human IgG2 Fc domain variant has up to 12, 11, 10, 9, 8, 7, 6, 5 or 4 or fewer deletions in total as compared to wild-type human IgG2 sequence (see, *e.g.*, SEQ ID NO: 162 below).

SEQ ID NO: 162:

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ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSD
GSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPG
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[0143] When the Fc domain variant is an IgG4 Fc domain variant, in some embodiments, such Fc domain variant comprises a S228P mutation (as designated according to Kabat, et al. (1991)). In some instances, a human IgG4 Fc domain variant has up to 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutation(s) in total as compared to wild-type human IgG4 sequence. In some embodiments, the Fc domain variant comprises a human IgG4 Fc sequence comprising one or more of S228P, E233P, F234V, L235A, and delG236 amino acid substitutions (as designated according to the EU numbering system per Kabat, et al. (1991)). In some embodiments, the Fc domain variant comprises a human IgG4 Fc sequence comprising one or more of S228P, E233P, F234V, L235A, delG236, and N297A amino acid substitutions (as designated according to the

EU numbering system per Kabat, et al. (1991).

[0144] In some embodiments, the Fc domain variant includes at least one of the mutations L234A, L235A, G237A or N297A of an IgG1 Fc region or at least one of the mutations A330S, P331S or N297A of an IgG2 Fc region. In some embodiments, the Fc domain variant includes at least two of the mutations L234A, L235A, G237A or N297A of an IgG1 Fc region or at least two of the mutations A330S, P331S or N297A of an IgG2 Fc region. In some embodiments, the Fc domain variant includes at least three of the mutations L234A, L235A, G237A or N297A of an IgG1 Fc region or consists of the mutations A330S, P331S and N297A of an IgG2 Fc region. In some embodiments, the Fc domain variant consists of the mutations L234A, L235A, G237A and N297A.

[0145] In some embodiments, the Fc domain variant exhibits reduced binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc domain variant exhibits ablated binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc domain variant exhibits a reduction of phagocytosis compared to the wild-type human IgG Fc region. In some embodiments, the Fc domain variant exhibits ablated phagocytosis compared to the wild-type human IgG Fc region.

[0146] SEQ ID NO: 88 and SEQ ID NO: 89 provide amino acid sequences of Fc domain IgG1 and IgG2 heavy chain constant regions. In some embodiments, an Fc domain variant is any variant of SEQ ID NOs: 90-95 as shown in *Table 7*.

Table 7. Amino Acid Sequences of Fc Domain Variants

SEQ ID NO:	AMINO ACID SEQUENCE
88	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL

SEQ ID NO:	AMINO ACID SEQUENCE
	VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG GNVFSCSVMHEALHNHYTQKSLSLSPGK
89	STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKTKVERKCCV ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPMLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
90	DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK
91	DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPG
92	VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN WYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWLNGKEYKCKVSN KGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPMLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
93	VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN WYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWLNGKEYKCKVSN

SEQ ID NO:	AMINO ACID SEQUENCE
	KGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPMLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPG
94	ERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWLNGKEYK CKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPMLDSGDSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
95	ERKSSVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWLNGKEYK CKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPMLDSGDSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG

[0147] Antibody-dependent cell-mediated cytotoxicity, which is also referred to herein as ADCC, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, Natural Killer (NK) cells and neutrophils) enabling these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell. Antibody-dependent cell-mediated phagocytosis, which is also referred to herein as ADCP, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain phagocytic cells (*e.g.*, macrophages) enabling these phagocytic effector cells to bind specifically to an antigen-bearing target cell and subsequently engulf and digest the target cell. Ligand-specific high-affinity IgG antibodies directed to the surface of target cells can stimulate the cytotoxic or phagocytic cells and can be used for such killing. In some embodiments, polypeptide constructs comprising an Fc domain variant or Fc domain dimer variant as described herein exhibit reduced ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs

comprising an Fc domain variant or Fc domain dimer variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in ADCC or ADCP compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs comprising an Fc domain variant or Fc domain dimer variant as described herein exhibit ablated ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region.

[0148] Complement-directed cytotoxicity, which is also referred to herein as CDC, refers to a form of cytotoxicity in which the complement cascade is activated by the complement component C1q binding to antibody Fc domains. In some embodiments, polypeptide constructs comprising an Fc domain variant or Fc domain dimer variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in C1q binding compared to a polypeptide construct comprising a wild-type Fc region. In some cases, polypeptide constructs comprising an Fc domain variant or Fc domain dimer variant as described herein exhibit reduced CDC as compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs comprising an Fc domain variant or Fc domain dimer variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in CDC compared to a polypeptide construct comprising a wild-type Fc region. In some cases, polypeptide constructs comprising an Fc domain variant or Fc domain dimer variant as described herein exhibit negligible CDC as compared to a polypeptide construct comprising a wild-type Fc region.

[0149] Fc domain variants or Fc domain dimer variants herein include those that exhibit reduced binding to an Fcγ receptor compared to the wild-type human IgG Fc region. For example, in some embodiments, an Fc domain variant or Fc domain dimer variant exhibits binding to an Fcγ receptor that is less than the binding exhibited by a wild-type human IgG Fc region to an Fcγ receptor, as described in the Examples. In some instances, an Fc domain variant or Fc domain dimer variant has reduced binding to an Fcγ receptor by a factor of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (fully ablated effector function). In some embodiments, the reduced binding is for any one or more Fcγ receptor, *e.g.*, CD16a, CD32a, CD32b, CD32c, or CD64.

[0150] In some instances, the Fc domain variants or Fc domain dimer variants disclosed

herein exhibit a reduction of phagocytosis compared to its wild-type human IgG Fc region. Such Fc domain variants or Fc domain dimer variants exhibit a reduction in phagocytosis compared to its wild-type human IgG Fc region, wherein the reduction of phagocytosis activity is *e.g.*, by a factor of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%. In some instances, an Fc domain variant or Fc domain dimer variant exhibits ablated phagocytosis compared to its wild-type human IgG Fc region.

[0151] In some embodiments, the Fc domain variants or Fc domain dimer variants disclosed herein are coupled to one or more fusion partners. In some cases the fusion partner is a therapeutic moiety. In some cases, the fusion partner is selected to enable targeting of an expressed protein, purification, screening, display, and the like. In some embodiments, the fusion partner also affects the degree of binding to Fc receptors or the degree of phagocytosis reduction. As described herein, in some embodiments, when an Fc domain variant or Fc domain dimer variant is coupled to a fusion partner, it forms a polypeptide construct as described below.

[0152] In some embodiments, fusion partners are linked to the Fc domain variant or Fc domain dimer variant sequence via a linker sequence. In some embodiments, the linker sequence generally comprises a small number of amino acids, such as less than ten amino acids, although longer linkers are also utilized. In some cases, the linker has a length less than 10, 9, 8, 7, 6, or 5 amino acids or shorter. In some cases, the linker has a length of at least 10, 11, 12, 13, 14, 15, 20, 25, 30, or 35 amino acids or longer. Optionally, in some embodiments, a cleavable linker is employed.

[0153] In some embodiments, a fusion partner is a targeting or signal sequence that directs an Fc domain variant or Fc domain dimer variant protein and any associated fusion partners to a desired cellular location or to the extracellular media. In some embodiments, certain signaling sequences target a protein to be either secreted into the growth media, or into the periplasmic space, located between the inner and outer membrane of the cell. In some embodiments, a fusion partner is a sequence that encodes a peptide or protein that enables purification or screening. Such fusion partners include, but are not limited to, polyhistidine tags (His-tags) (for example His6 (SEQ ID NO: 223) and His10 (SEQ ID NO: 224)) or other tags for use with Immobilized Metal Affinity Chromatography (IMAC) systems (*e.g.*, Ni⁺² affinity columns), GST fusions, MBP fusions, Strep-tag, the BSP biotinylation target sequence of the bacterial enzyme BirA, and

epitope tags which are targeted by antibodies (for example c-myc tags, flag-tags, and the like).

[0154] In some embodiments, such tags are useful for purification, for screening, or both. For example, in some embodiments, an Fc domain variant or Fc domain dimer variant is purified using a His-tag by immobilizing it to a Ni²⁺ affinity column, and then after purification the same His-tag is used to immobilize the antibody to a Ni²⁺ coated plate to perform an ELISA or other binding assay as described elsewhere herein. In some embodiments, a fusion partner enables the use of a selection method to screen Fc domain variants or Fc domain dimer variants as described herein.

[0155] Various fusion partners that enable a variety of selection methods are available. For example, by fusing the members of an Fc domain variant or Fc domain dimer variant library to the gene III protein, phage display can be employed. In some embodiments, fusion partners Fc domain variants or Fc domain dimer variants to be labeled. Alternatively, in some embodiments, a fusion partner binds to a specific sequence on the expression vector, enabling the fusion partner and associated Fc domain variant or Fc domain dimer variant to be linked covalently or noncovalently with the nucleic acid that encodes them.

[0156] In some embodiments, when a fusion partner is a therapeutic moiety, the therapeutic moiety is, *e.g.*, a peptide, a protein, an antibody, a siRNA, or a small molecule. Non-limiting examples of therapeutic antibodies that are coupled to the Fc domain variants or Fc domain dimer variants of the present disclosure include, but are not limited to antibodies that recognize CD47. Non-limiting examples of therapeutic polypeptides that are coupled to the Fc domain variants or Fc domain dimer variants of the present disclosure include, but are not limited to, CD47 binding polypeptides, including SIRPα polypeptides. In such instances, the CD47 binding polypeptide is attached or fused to an Fc domain variant or Fc domain dimer variant of the disclosure. Examples of CD47 binding polypeptides include, but are not limited to, anti-CD47 antibodies or fragments thereof, and ligands of CD47 such as SIRPα or a fragment thereof. Additional examples of CD47 binding polypeptides include, but are not limited to naturally-occurring forms of SIRPα as well as mutants thereof.

[0157] In some embodiments, disclosed herein is a polypeptide comprising an Fc domain dimer variant, wherein the Fc domain dimer variant comprises two Fc domain variants, wherein each Fc domain variant independently is selected from (i) a human IgG1 Fc region consisting of

mutations L234A, L235A, G237A, and N297A; (ii) a human IgG2 Fc region consisting of mutations A330S, P331S and N297A; or (iii) a human IgG4 Fc region comprising mutations S228P, E233P, F234V, L235A, delG236, and N297A. In some embodiments, the Fc domain variants are identical (*i.e.*, homodimer). In some embodiments, the Fc domain variants are different (*i.e.*, heterodimer). In some embodiments, at least one of the Fc domain variant in an Fc domain dimer is a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A. In some embodiments, at least one of the Fc domain variants in an Fc domain dimer is a human IgG2 Fc region consisting of mutations A330S, P331S and N297A. In some embodiments, the Fc domain dimer variant exhibits ablated or reduced binding to an Fcγ receptor compared to the wild-type version of the human IgG Fc region. In some embodiments, the Fc domain dimer variant exhibits ablated or reduced binding to CD16a, CD32a, CD32b, CD32c, and CD64 Fcγ receptors compared to the wild-type version of the human IgG Fc region. In some embodiments, the Fc domain dimer variant exhibits ablated or reduced binding to C1q compared to the wild-type version of the human IgG Fc fusion. In some embodiments, at least one of the Fc domain variants in an Fc domain dimer variant is a human IgG4 Fc region comprising mutations S228P, E233P, F234V, L235A, delG236, and N297A. In some embodiments, the Fc domain dimer variant exhibits ablated or reduced binding to an Fcγ receptor compared to the wild-type human IgG4 Fc region. In some embodiments, the Fc domain dimer variant exhibits ablated or reduced binding to CD16a and CD32b Fcγ receptors compared to the wild-type version of its human IgG4 Fc region. In some embodiments, the Fc domain dimer variant binds to an Fcγ receptor with a KD greater than about 5×10^{-6} M.

[0158] In some embodiments, the Fc domain dimer variant further comprises a CD47 binding polypeptide. In some embodiments, the Fc domain dimer variant exhibits ablated or reduced binding to an Fcγ receptor compared to a wild-type version of a human IgG Fc region. In some embodiments, the CD47 binding polypeptide does not cause acute anemia in rodents and non-human primates. In some embodiments, the CD47 binding polypeptide does not cause acute anemia in humans.

[0159] In some embodiments, the CD47 binding polypeptide is a signal-regulatory protein α (SIRP-α) polypeptide or a fragment thereof. In some embodiments, the SIRPα polypeptide comprises a SIRPα D1 domain variant comprising the amino acid sequence, EEELQX₁IQPDKSVLVAAGETATLRCTX₂TSLX₃PVGPIQWFRGAGPGRX₄LIYNQX₅EGX₆

FPRVTTVSDX₇TKRNNMDFSIRIGX₈ITPADAGTYXCX₉KFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 221), wherein X₁ is V or I; X₂ is A or I; X₃ is I or F; X₄ is E or V; X₅ is K or R; X₆ is H or P; X₇ is L or T; X₈ is any amino acid other than N; and X₉ is V or I. In some embodiments, the SIRP α polypeptide comprises a SIRP α D1 domain variant wherein X₁ is V or I; X₂ is A or I; X₃ is I or F; X₄ is E; X₅ is K or R; X₆ is H or P; X₇ is L or T; X₈ is not N; and X₉ is V.

[0160] In some embodiments, disclosed herein, is a polypeptide comprising: a SIRP α D1 domain variant, wherein the SIRP α D1 domain variant is a non-naturally occurring high affinity SIRP α D1 domain, wherein the SIRP α D1 domain variant binds to human CD47 with an affinity that is at least 10-fold greater than the affinity of a naturally occurring D1 domain; and an Fc domain variant, wherein the Fc domain variant is linked to a second polypeptide comprising a second Fc domain variant to form an Fc domain dimer variant, wherein the Fc domain dimer variant has ablated or reduced effector function. In some embodiments, the non-naturally occurring high affinity SIRP α D1 domain comprises an amino acid mutation at residue 80.

[0161] In some embodiments, disclosed herein, is a SIRP α D1 domain variant, wherein the SIRP α D1 domain variant binds CD47 from a first species with a K_D less than 250 nM; and wherein the SIRP α D1 domain variant binds CD47 from a second species with a K_D less than 250 nM; and the K_D for CD47 from the first species and the K_D for CD47 from the second species are within 100 fold of each other; wherein the first species and the second species are selected from the group consisting of: human, rodent, and non-human primate. In some embodiments, the SIRP α D1 domain variant binds CD47 from at least 3 different species. In some embodiments, the non-human primate is cynomolgus monkey.

[0162] In some embodiments, disclosed herein, is a polypeptide comprising (a) a SIRP α D1 domain that binds human CD47 with a K_D less than 250 nM; and (b) an Fc domain or variant thereof linked to the N-terminus or the C-terminus of the SIRP α D1 domain, wherein the polypeptide does not cause acute anemia in rodents and non-human primates. In some embodiments, the polypeptide is a non-naturally occurring variant of a human SIRP- α . In some embodiments, administration of the polypeptide in vivo results in hemoglobin reduction by less than 50% during the first week after administration. In some embodiments, administration of the polypeptide in humans results in hemoglobin reduction by less than 50% during the first week

after administration. In some embodiments, the polypeptide further comprises at least one Fc domain dimer variant, wherein the Fc domain dimer variant comprises an Fc domain variant selected from (i) a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A; (ii) a human IgG2 Fc region consisting of mutations A330S, P331S and N297A; or (iii) a human IgG4 Fc region comprising mutations S228P, E233P, F234V, L235A, delG236, and N297A. In some embodiments, the Fc domain variant is a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A. In some embodiments, the Fc domain variant is a human IgG2 Fc region consisting of mutations A330S, P331S and N297A.

[0163] The SIRP α constructs of the disclosure include a SIRP α domain or variant thereof that has its C-terminus joined to the N-terminus of an Fc domain or variant thereof by way of a linker using conventional genetic or chemical means, *e.g.*, chemical conjugation. In some embodiments, a linker (*e.g.*, a spacer) is inserted between the polypeptide and the Fc domain or variant thereof. In some embodiments, a polypeptide of the disclosure including a SIRP α D1 domain variant is fused to an Fc domain variant that is incapable of forming a dimer. In some embodiments, a polypeptide of the disclosure is fused to an Fc domain or variant thereof that is capable of forming a dimer, *e.g.*, a heterodimer, with another Fc domain or variant thereof. In some embodiments, a polypeptide of the invention is fused to an Fc domain or variant thereof and this fusion protein forms a homodimer. In some embodiments, a polypeptide of the disclosure is fused to a first Fc domain or variant thereof and a different protein or peptide (*e.g.*, an antibody variable region) is fused to a second Fc domain or variant thereof. In some embodiments, a SIRP α D1 domain or variant thereof is joined to a first Fc domain or variant thereof and a therapeutic protein (*e.g.*, a cytokine, an interleukin, an antigen, a steroid, an anti-inflammatory agent, or an immunomodulatory agent) is joined to a second Fc domain or variant thereof. In some embodiments, the first and second Fc domains or variants thereof form a heterodimer.

[0164] Without limiting the foregoing, in some embodiments, a SIRP α D1 domain variant polypeptide (*e.g.*, any of the variants described in Tables 2, 5, and 6) is fused to an Fc polypeptide or Fc variant polypeptide, such as an Fc domain or variant thereof. Examples of polypeptides comprising a SIRP α D1 domain variant polypeptide and a fused Fc domain variant polypeptide include, but are not limited to, SEQ ID NOS: 96-137, 214, and 216 shown in **Table 8**.

Table 8. Polypeptides Comprising SIRPa D1 Domain Variants Fused to Fc Domain Variants

SEQ ID NO:	Amino Acid Sequence
96	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRTTVSDTTKRNNMDFSIRIGNITPADAGTYCYKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
97	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRTTVSDLTNRNNMDFSIRIGNITPADAGTYCYKFRKGSPDDVEF KSGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
98	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYCYKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
99	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRTTVSDLTNRNNMDFSIRIGAITPADAGTYCYKFRKGSPDDVEF KSGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM

SEQ ID NO:	Amino Acid Sequence
	TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
100	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ REGPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEF KSGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
101	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFK SGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
102	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
103	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFK SGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK

SEQ ID NO:	Amino Acid Sequence
	NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
104	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
105	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTY YCIKFRKGSPDDVEFKS GAGTELSVRAKPSVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVS VLT VVHQDWL NGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDS DGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
106	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRVLIYNQ RQGPFPRVTTVSDLT KRNNMDFSIRIGNITPADAGTY YCVKFRKGSPDDVEF KSGAGTELSVRAKPSVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVS VLT VVHQD WLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDS DGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
107	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCIKFRKGSPDDVEFKS GAGTELSVRAKPSVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVS VLT VVHQDWL NGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT

SEQ ID NO:	Amino Acid Sequence
	CLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
108	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEF KSGAGTELSVRAKPSVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQD WLNKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
109	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ REGPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEF KSGAGTELSVRAKPSVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQD WLNKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
110	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPSVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQD WLNKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
111	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFKS GAGTELSVRAKPSVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWL NGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT

SEQ ID NO:	Amino Acid Sequence
	CLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
112	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFK SGAGTELSVRAKPSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQD WLNKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
113	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFKS GAGTELSVRAKPSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWL NGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
114	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKS GAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVV HQDWLNKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
115	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRVTTVSDLTNRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEF KSGAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVL TVVHQDWLNKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMT

SEQ ID NO:	Amino Acid Sequence
	KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
116	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVV HQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
117	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRVTTVSDLTNRNNMDFSIRIGAITPADAGTYCYVKFRKGSPDDVEF KSGAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPE EVTCTVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTV VHVDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTK KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
118	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ REGPFPRVTTVSDLTNRNNMDFSIRIGAITPADAGTYCYVKFRKGSPDDVEF KSGAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPE EVTCTVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTV VHVDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTK KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
119	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDLTNRNNMDFSIRIGAITPADAGTYCYVKFRKGSPDDVEFK SGAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTV VHVDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTK

SEQ ID NO:	Amino Acid Sequence
	NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
120	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDLTNRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFKS GAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVV HQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
121	EEELQVIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTNRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFK SGAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTV VHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
122	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTNRNNMDFSIRIGAITPADAGTYYCVKFRKGSPDDVEFKS GAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVV HQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
123	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTNRNNMDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK

SEQ ID NO:	Amino Acid Sequence
	NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
124	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
125	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
126	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSERKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVV HQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDS DGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
127	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSERKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVV HQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKN

SEQ ID NO:	Amino Acid Sequence
	QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
128	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGFPRVTTVSDTTKRNNMDFSIIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSERKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVV HQDWLNGKEYKCKVSNKGLPAIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
129	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGFPRVTTVSDTTKRNNMDFSIIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSERKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVVSVLTVV HQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
130	EEELQVIQPDKSVSVAAGESAILHCTVTSLIPVGPIQWFRGAGPARELIYNQK EGHFPRVTTVSESTKRENMDFSISISNITPADAGTYCYCVKFRKGSPDTEFKSG AGTELSVRAKPSSESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
131	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIIRIGAITPADAGTYCYCVKFRKGSPDDVEFKS GAGTELSVRAKPSSESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK

SEQ ID NO:	Amino Acid Sequence
	NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV DKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK
132	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFKS GAGTELSVRAKPSESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV DKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK
133	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFKS GAGTELSVRAKPSESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV DKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK
134	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTY YCIKFRKGSPDDVEFKS GAGTELSVRAKPSAAAPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV KSRWQEGNVFSCSVMEALHNHYTQKSLSLSPGK
135	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ REGPFPRVTTVSDLTNRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEF KSGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM

SEQ ID NO:	Amino Acid Sequence
	TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
136	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
137	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCIKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
211	EEELQIIQPDKSVLVAAGETATLRCTITSLRPVGPIQWFRGAGPGRELIYNQR DGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGIPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
214	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ REGPFPRVTTVSDLTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEF KSGAGTELSVRAKPSERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFASTFRVSVLTVL VHQQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMT

SEQ ID NO:	Amino Acid Sequence
	KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
216	EEELQIIQPKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYCYCIKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSFGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
217	EEELQIIQPKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRELIYNQRE GPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTYCYCVKFRKGSPDDVEFKS GAGTELSVRAKPSDKTHTCPECPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCEVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSFGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0165] In some embodiments, the polypeptide comprises a SIRP α D1 variant domain that has at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to any variant provided in *Table 8*.

[0166] In some embodiments, the polypeptide comprises a SIRP α D1 domain variant that has at least 85% sequence identity (*e.g.*, at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to SEQ ID NOs: 98-104, 107-113, 116-122, or 135-137 in *Table 8*.

[0167] In some embodiments, the polypeptide comprises (a) a signal-regulatory protein α (SIRP- α) D1 variant, wherein the SIRP α D1 domain variant comprises the amino acid sequence, EEX₁X₂QX₃IQPKDX₄VX₅VAAGEX₆X₇X₈LX₉CTX₁₀TSLX₁₁PVGPIQWFRGAGPX₁₂RX₁₃LIY
NQX₁₄X₁₅GX₁₆FPRVTTVSX₁₇X₁₈TX₁₉RX₂₀NMDFX₂₁IX₂₂IX₂₃X₂₄ITX₂₅ADAGTYCYCX₂₆KX₂₇

RKGSPDX₂₈X₂₉EX₃₀KSGAGTELSVRX₃₁KPS (SEQ ID NO: 47), wherein X₁ is E, or G; X₂ is L, I, or V; X₃ is V, L, or I; X₄ is S, or F; X₅ is L, or S; X₆ is S, or T; X₇ is A, or V; X₈ is I, or T; X₉ is H, R, or L; X₁₀ is A, V, I, or L; X₁₁ is I, T, S, or F; X₁₂ is A, or G; X₁₃ is E, V, or L; X₁₄ is K, or R; X₁₅ is E, or Q; X₁₆ is H, P, or R; X₁₇ is D, or E; X₁₈ is S, L, T, or G; X₁₉ is K, or R; X₂₀ is E, or N; X₂₁ is S, or P; X₂₂ is S, or R; X₂₃ is S, or G; X₂₄ is any amino acid; X₂₅ is any amino acid; X₂₆ is V, or I; X₂₇ is F, L, or V; X₂₈ is D or absent; X₂₉ is T, or V; X₃₀ is F, or V; and X₃₁ is A, or G; and wherein the SIRPα D1 domain variant comprises at least two amino acid substitutions relative to a wild-type SIRPα D1 domain having a sequence according to any one of SEQ ID NOs: 1 to 10; and (b) an Fc domain dimer variant having two Fc domain variants, wherein each Fc domain variant independently is (i) a human IgG1 Fc region comprising a N297A mutation; (ii) a human IgG1 Fc region comprising L234A, L235A, and G237A mutations; (iii) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations; (iv) a human IgG2 Fc region comprising a N297A mutation; (v) a human IgG2 Fc region comprising A330S and P331S mutations; (vi) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations; (vii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations; or (viii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations.

[0168] In some embodiments, the polypeptide comprises a SIRPα D1 domain variant wherein the SIRPα D1 domain variant comprises an amino acid sequence according to SEQ ID NO: 47; an Fc domain dimer having two Fc domains, wherein one of the Fc domains is an Fc domain variant comprising a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations.

Dimerization of Fc domains

[0169] In some embodiments, a SIRPα D1 domain variant polypeptide (*e.g.*, any of the variants described in Tables 2, 5, and 6) is fused to a first Fc domain (*e.g.*, an Fc domain variant) either at the N-terminus or at the C-terminus. In some embodiments, the first Fc domain is a variant that is incapable of forming a dimer. In some embodiments, the first Fc domain forms a dimer with a second Fc domain. In some embodiments, the first and second Fc domains comprise amino acid substitutions that promote heterodimerization between the first and second domain Fc domains.

[0170] In some embodiments, each of the two Fc domains in an Fc domain dimer includes amino acid substitutions that promote the heterodimerization of the two monomers. In some embodiments, a SIRP α construct is formed, for example, from a first subunit including a SIRP α D1 domain variant polypeptide fused to a first Fc domain and a second subunit including a second Fc domain (*e.g.*, without a SIRP α D1 domain variant polypeptide or any other polypeptide). In some embodiments, a construct has a single SIRP α D1 domain variant polypeptide linked to an Fc domain dimer (*e.g.*, single arm). In some embodiments, a construct has two SIRP α D1 domain variant polypeptides linked to an Fc domain dimer (*e.g.*, double arm). In some embodiments, a SIRP α D1 domain variant having a K_D of about 500 nM is particularly useful in a double arm construct. In some embodiments, a SIRP α D1 domain variant having a K_D of about 50 nM is particularly useful in a double arm construct. In some embodiments, a SIRP α D1 domain variant having a K_D of about 5 nM is useful in a double arm construct and a single arm construct. In some embodiments, a SIRP α D1 domain variant having a K_D of about 500 pM is useful in a double arm construct and a single arm construct. In some embodiments, a SIRP α D1 domain variant having a K_D of about 100 pM is useful in a double arm construct and a single arm construct. In some embodiments, a SIRP α D1 domain variant having a K_D of about 50 pM is useful in a double arm construct and a single arm construct. In some embodiments, a SIRP α D1 domain variant having a K_D of about 10 pM is useful in a double arm construct and a single arm construct.

[0171] In some embodiments, heterodimerization of Fc domains is promoted by introducing different, but compatible, substitutions in the two Fc domains, such as “knob-into-hole” residue pairs and charge residue pairs. The knob and hole interaction favors heterodimer formation, whereas the knob-knob and the hole-hole interaction hinder homodimer formation due to steric clash and deletion of favorable interactions. A hole refers to a void that is created when an original amino acid in a protein is replaced with a different amino acid having a smaller side-chain volume. A knob refers to a bump that is created when an original amino acid in a protein is replaced with a different amino acid having a larger side-chain volume. For example, in some embodiments, an amino acid being replaced is in the CH3 antibody constant domain of an Fc domain and involved in the dimerization of two Fc domains. In some embodiments, a hole in one CH3 antibody constant domain is created to accommodate a knob in another CH3 antibody constant domain, such that the knob and hole amino acids act to promote or favor the

heterodimerization of the two Fc domains. In some embodiments, a hole in one CH3 antibody constant domain is created to better accommodate an original amino acid in another CH3 antibody constant domain. In some embodiments, a knob in one CH3 antibody constant domain is created to form additional interactions with original amino acids in another CH3 antibody constant domain.

[0172] In some embodiments, a hole is constructed by replacing amino acids having larger side chains such as tyrosine or tryptophan with amino acids having smaller side chains such as alanine, valine, or threonine, for example a Y407V mutation in the CH3 antibody constant domain. Similarly, in some embodiments, a knob is constructed by replacing amino acids having smaller side chains with amino acids having larger side chains, for example a T366W mutation in the CH3 antibody constant domain. In some embodiments, one Fc domain includes the knob mutation T366W and the other Fc domain includes hole mutations T366S, L358A, and Y407V. In some embodiments, a polypeptide of the disclosure including a SIRP α D1 domain variant is fused to an Fc domain including the knob mutation T366W to limit unwanted knob-knob homodimer formation. Examples of knob-into-hole amino acid pairs are included, without limitation, in *Table 9* and examples of knob-into-hole Fc domain variants and SIRP α – Fc fusions are provided in *Table 10*.

Table 9. Knob-Into-Hole Amino Acid Pairs

First Fc Domain	Y407T	Y407A	F405A	T394S	T366S L358A Y407V	T394W Y407T	T394S Y407A	T366W T394S
Second Fc Domain	T366Y	T366W	T394W	F405W	T366W	T366Y F405A	T366W F405W	F405W Y407A

Table 10. Exemplary Fc Domain Variants and SIRPa D1 Domain Variant – Fc Domain Variant Fusion Polypeptides

SEQ ID NO:	Amino Acid Sequence
138	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYCYCIKFRKGGSPDDVEFK SGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSVMSVMEALHNHYTQKSLSLSPGK
139	DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNV FSVMSVMEALHNHYTQKSLSLSPGK
140	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQR QGPFPRTTVSDTTKRNNMDFSIRIGAITPADAGTYCYCIKFRKGGSPDDVEFK SGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSK LTVDKSRWQQGNVFSVMSVMEALHNHYTQKSLSLSPGK
141	DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSVMSVMEALHNHYTQKSLSLSPGK

SEQ ID NO:	Amino Acid Sequence
142	EEELQHQPDKSVLVAAGETATLRCITITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
143	EEELQHQPDKSVLVAAGETATLRCITITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
144	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVI WSGGNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTY DYEFAYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCRKTHTCPRCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL YSRLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
145	EEELQHQPDKSVLVAAGETATLRCITITSLFPVGPIQWFRGAGPGRELIYNQR EGPFPRVTTVSDTTKRNNMDFSIRIGAITPADAGTY YCVKFRKGSPDDVEFK SGAGTELSVRAKPSEKTHTCPECPAPEAAGAPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT

SEQ ID NO:	Amino Acid Sequence
	KNQVSLTCEVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
146	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTY YCVKFRKGSPDDVEF KSGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
147	DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
148	EEELQVIQPDKSVLVAAGETATLRCTATSLFPVGPIQWFRGAGPGRELIYNQ RQGPFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTY YCVKFRKGSPDDVEF KSGAGTELSVRAKPSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
149	DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0173] In addition to the knob-into-hole strategy, in some embodiments, electrostatic steering

is also used to control the dimerization of Fc domains. Electrostatic steering refers to the utilization of favorable electrostatic interactions between oppositely charged amino acids in peptides, protein domains, and proteins to control the formation of higher ordered protein molecules. In particular, to control the dimerization of Fc domains using electrostatic steering, one or more amino acid residues that make up the CH3-CH3 interface are replaced with positively- or negatively-charged amino acid residues such that the interaction becomes electrostatically favorable or unfavorable depending on the specific charged amino acids introduced. In some embodiments, a positively-charged amino acid in the interface, such as lysine, arginine, or histidine, is replaced with a negatively-charged amino acid such as aspartic acid or glutamic acid. In some embodiments, a negatively-charged amino acid in the interface is replaced with a positively-charged amino acid. In some embodiments, the charged amino acids are introduced to one of the interacting CH3 antibody constant domains, or both. In some embodiments, introducing charged amino acids to the interacting CH3 antibody constant domains of the two Fc domains promotes the selective formation of heterodimers of Fc domains as controlled by the electrostatic steering effects resulting from the interaction between charged amino acids. Examples of electrostatic steering amino acid pairs are included, without limitation, in *Table 11*.

Table 11. Electrostatic Steering Amino Acid Pairs

Fc domain monomer 1	K409D	K409D	K409E	K409E	K392D	K392D	K392E	K392E	K409D K392D	K370E K409D K439E
Fc domain monomer 2	D399K	D399R	D399K	D399R	D399K	D399R	D399K	D399R	D399K D356K	D356K E357K D399K

[0174] Other methods used to control the heterodimerization of Fc domains, especially in the context of constructing a bispecific antibody, are available.

[0175] In some embodiments, a first Fc domain and a second Fc domain each includes one or more of the following amino acid substitutions: T366W, T366S, L368A, Y407V, T366Y, T394W, F405W, Y349T, Y349E, Y349V, L351T, L351H, L351N, L351K, P353S, S354D, D356K, D356R, D356S, E357K, E357R, E357Q, S364A, T366E, L368T, L368Y, L368E, K370E, K370D, K370Q, K392E, K392D, T394N, P395N, P396T, V397T, V397Q, L398T, D399K, D399R, D399N, F405T, F405H, F405R, Y407T, Y407H, Y407I, K409E, K409D, K409T, and K409I, relative to the sequence of human IgG1.

[0176] In some embodiments an Fc domain comprises: (a) one of the following amino acid substitutions relative to wild type human IgG1: T366W, T366S, L368A, Y407V, T366Y, T394W, F405W, Y349T, Y349E, Y349V, L351T, L351H, L351N, L351K, P353S, S354D, D356K, D356R, D356S, E357K, E357R, E357Q, S364A, T366E, L368T, L368Y, L368E, K370E, K370D, K370Q, K392E, K392D, T394N, P395N, P396T, V397T, V397Q, L398T, D399K, D399R, D399N, F405T, F405H, F405R, Y407T, Y407H, Y407I, K409E, K409D, K409T, or K409I; or (b) (i) a N297A mutation relative to a human IgG1 Fc region; (ii) a L234A, L235A, and G237A mutation relative to a human IgG1 Fc region; (iii) a L234A, L235A, G237A, and N297A mutation relative to a human IgG1 Fc region; (iv) a N297A mutation relative to a human IgG2 Fc region; (v) a A330S and P331S mutation relative to a human IgG2 Fc region; (vi) a A330S, P331S, and N297A mutation relative to a human IgG2 Fc region; (vii) a S228P, E233P, F234V, L235A, and delG236 mutation relative to a human IgG4 Fc region; or (viii) a S228P, E233P, F234V, L235A, delG236, and N297A mutation relative to a human IgG4 Fc region. In some embodiments an Fc domain variant comprises: (a) one of the following amino acid substitutions relative to wild type human IgG1: T366W, T366S, L368A, Y407V, T366Y, T394W, F405W, Y349T, Y349E, Y349V, L351T, L351H, L351N, L351K, P353S, S354D, D356K, D356R, D356S, E357K, E357R, E357Q, S364A, T366E, L368T, L368Y, L368E, K370E, K370D, K370Q, K392E, K392D, T394N, P395N, P396T, V397T, V397Q, L398T, D399K, D399R, D399N, F405T, F405H, F405R, Y407T, Y407H, Y407I, K409E, K409D, K409T, or K409I; and (b) further comprises (i) a N297A mutation relative to a human IgG1 Fc region; (ii) a L234A, L235A, and G237A mutation relative to a human IgG1 Fc region; (iii) a L234A, L235A, G237A, and N297A mutation relative to a human IgG1 Fc region; (iv) a N297A mutation relative to a human IgG2 Fc region; (v) a A330S and P331S mutation relative to a human IgG2 Fc region; (vi) a A330S, P331S, and N297A mutation relative to a human IgG2 Fc

region; (vii) a S228P, E233P, F234V, L235A, and delG236 mutation relative to a human IgG4 Fc region; or (viii) a S228P, E233P, F234V, L235A, delG236, and N297A mutation relative to a human IgG4 Fc region.

[0177] In some embodiments, the first and second Fc domains include different amino acid substitutions. In some embodiments, the first Fc domain includes T366W. In some embodiments, the second Fc domain includes T366S, L368A, and Y407V. In some embodiments, the first Fc domain includes D399K. In some embodiments, the second Fc domain includes K409D.

Linkers

[0178] Disclosed herein, in some embodiments, are polypeptides comprising a signal-regulatory protein α (SIRP- α) D1 variant comprising a SIRP α D1 domain, or a fragment thereof, having an amino acid mutation at residue 80 relative to a wild-type SIRP α D1 domain; and at least one additional amino acid mutation relative to a wild-type SIRP α D1 domain at a residue selected from the group consisting of: residue 6, residue 27, residue 31, residue 47, residue 53, residue 54, residue 56, residue 66, and residue 92.

[0179] Also disclosed herein, in some embodiments, are polypeptides comprising an Fc variant, wherein the Fc variant comprises an Fc domain dimer comprising two Fc domain variants, wherein each Fc domain variant independently is selected from (i) a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A; (ii) a human IgG2 Fc region consisting of mutations A330S, P331S and N297A; or (iii) a human IgG4 Fc region comprising mutations S228P, E233P, F234V, L235A, delG236, and N297A.

[0180] In the present disclosure, a linker is used to describe a linkage or connection between polypeptides or protein domains or associated non-protein moieties. In some embodiments, a linker is a linkage or connection between an Fc domain (or variant thereof) and a SIRP α D1 domain variant. In some embodiments, the linker connects the C-terminus of the SIRP α D1 domain variant and the N-terminus of the Fc domain variant, such that the two polypeptides are joined to each other in tandem series.

[0181] In some embodiments, a linker is a simple covalent bond, *e.g.*, a peptide bond, a synthetic polymer, or any kind of bond created from a chemical reaction, *e.g.* chemical

conjugation. When a linker is a peptide bond, in some embodiments, the carboxylic acid group at the C-terminus of one protein domain reacts with the amino group at the N-terminus of another protein domain in a condensation reaction to form a peptide bond. In some embodiments, the peptide bond is formed from synthetic means through a conventional organic chemistry reaction, or by natural production from a host cell, wherein a nucleic acid molecule encoding the DNA sequences of both proteins (*e.g.*, an Fc domain variant and a SIRP α D1 domain variant) in tandem series can be directly transcribed and translated into a contiguous polypeptide encoding both proteins by the necessary molecular machineries (*e.g.*, DNA polymerase and ribosome) in the host cell.

[0182] When a linker is a synthetic polymer, in some embodiments, the polymer is functionalized with reactive chemical functional groups at each end to react with the terminal amino acids at the connecting ends of two proteins.

[0183] When a linker (except peptide bond mentioned above) is made from a chemical reaction, in some embodiments, chemical functional groups (*e.g.*, amine, carboxylic acid, ester, azide, or other functional groups), are attached synthetically to the C-terminus of one protein and the N-terminus of another protein, respectively. In some embodiments, the two functional groups then react through synthetic chemistry means to form a chemical bond, thus connecting the two proteins together.

Spacers

[0184] In the present disclosure, in some embodiments, a linker between an Fc domain monomer and a SIRP α D1 variant polypeptide of the disclosure, is an amino acid spacer including about 1-200 amino acids. Suitable peptide spacers include peptide linkers containing flexible amino acid residues such as glycine and serine. Examples of linker sequences are provided in **Table 12**. In some embodiments, a spacer contains motifs, *e.g.*, multiple or repeating motifs, of GS, GG, GGS, GGG, GGGGS (SEQ ID NO: 163), GGSG (SEQ ID NO: 164), or SGGG (SEQ ID NO: 165). In some embodiments, a spacer contains 2 to 12 amino acids including motifs of GS, *e.g.*, GS, GSGS (SEQ ID NO: 166), GSGSGS (SEQ ID NO: 167), GSGSGSGS (SEQ ID NO: 168), GSGSGSGSGS (SEQ ID NO: 169), or GSGSGSGSGSGS (SEQ ID NO: 170). In some embodiments, a spacer contains 3 to 12 amino acids including motifs of GGS, *e.g.*, GGS, GGS GGS (SEQ ID NO: 171), GGS GGS GGS (SEQ ID NO: 172),

and GGS GGSGGSGGGS (SEQ ID NO: 173). In some embodiments, a spacer contains 4 to 12 amino acids including motifs of GGSG (SEQ ID NO: 164), *e.g.*, GGSG (SEQ ID NO: 164), GGSGGGSG (SEQ ID NO: 174), or GGSGGGSGGGSG (SEQ ID NO: 175). In some embodiments, a spacer contains motifs of GGGGS (SEQ ID NO: 163), *e.g.*, GGGGSGGGGSGGGGS (SEQ ID NO: 176). In some embodiments, a spacer contains amino acids other than glycine and serine, *e.g.*, AAS (SEQ ID NO: 177), AAAL (SEQ ID NO: 178), AAAK (SEQ ID NO: 179), AAAR (SEQ ID NO: 180), EGKSSGSGSESKST (SEQ ID NO: 181), GSAGSAAGSGEF (SEQ ID NO: 182), AEAAAKEAAAKA (SEQ ID NO: 183), KESGSVSSEQLAQFRSLD (SEQ ID NO: 184), GGGGAGGGG (SEQ ID NO: 185), GENLYFQSGG (SEQ ID NO: 186), SACYCELS (SEQ ID NO: 187), RSIAT (SEQ ID NO: 188), RPACKIPNDLKQKVMNH (SEQ ID NO: 189), GGSAGGSGSGSSGGSSGASGTGTAGGTGSGSGTGSG (SEQ ID NO: 190), AAANSSIDLISVPVDSR (SEQ ID NO: 191), or GGS GGGSEG GGGSEG GGGSEG GGGSEG GGGSEG GGGSGGGS (SEQ ID NO: 192).

[0185] In some embodiments, a spacer contains motifs, *e.g.*, multiple or repeating motifs, of EAAAK (SEQ ID NO: 193). In some embodiments, a spacer contains motifs, *e.g.*, multiple or repeating motifs, of proline-rich sequences such as (XP)_n, in which X is any amino acid (*e.g.*, A, K, or E) and n is from 1-5, and PAPAP (SEQ ID NO: 194).

Table 12. Linker Sequences

SEQ ID NO:	AMINO ACID SEQUENCE
163	GGGGS
164	GGSG
165	SGGG
166	GSGS
167	GSGSGS
168	GSGSGSGS

SEQ ID NO:	AMINO ACID SEQUENCE
169	GSGSGSGSGS
170	GSGSGSGSGSGS
171	GGSGGS
172	GGSGGSGGS
173	GGSGGSGGSGGS
174	GGSGGGSG
175	GGSGGGSGGGSG
176	GGGGSGGGGSGGGGS
177	AAS
178	AAAL
179	AAAK
180	AAAR
181	EGKSSSGSGSESKST
182	GSAGSAAGSGEF
183	AEAAAKEAAKA
184	KESGSVSSEQLAQFRSLD
185	GGGGAGGGG
186	GENLYFQSGG
187	SACYCELS

SEQ ID NO:	AMINO ACID SEQUENCE
188	RSIAT
189	RPACKIPNDLKQKVMNH
190	GGSAAGSGSGSSGGSSGASGTGTAGGTGSGSGTGSG
191	AAANSSIDLISVPVDSR
192	GGSGGGSEGGGSEGGGSEGGGSEGGGSEGGGSGGGS
193	EAAAK
194	PAPAP

[0186] In some embodiments, the length of the peptide spacer and the amino acids used is adjusted depending on the two proteins involved and the degree of flexibility desired in the final protein fusion polypeptide. In some embodiments, the length of the spacer is adjusted to ensure proper protein folding and avoid aggregate formation. In some embodiments, a spacer is A or AAAL (SEQ ID NO: 178).

Vectors, Host Cells, and Protein Production

[0187] Disclosed herein, in some embodiments, are polypeptides comprising a signal-regulatory protein α (SIRP- α) D1 variant comprising a SIRP α D1 domain, or a fragment thereof, having an amino acid mutation at residue 80 relative to a wild-type SIRP α D1 domain; and at least one additional amino acid mutation relative to a wild-type SIRP α D1 domain at a residue selected from the group consisting of: residue 6, residue 27, residue 31, residue 47, residue 53, residue 54, residue 56, residue 66, and residue 92.

[0188] Also disclosed herein, in some embodiments, are polypeptides comprising an Fc variant, wherein the Fc variant comprises an Fc domain dimer having two Fc domain monomers, wherein each Fc domain monomer independently is selected from (i) a human IgG1 Fc region consisting of mutations L234A, L235A, G237A, and N297A; (ii) a human IgG2 Fc region consisting of mutations A330S, P331S and N297A; or (iii) a human IgG4 Fc region comprising

mutations S228P, E233P, F234V, L235A, delG236, and N297A.

[0189] In some embodiments, the polypeptides of the disclosure are produced from a host cell. A host cell refers to a vehicle that includes the necessary cellular components, *e.g.*, organelles, needed to express the polypeptides and fusion polypeptides described herein from their corresponding nucleic acids. In some embodiments, the nucleic acids are included in nucleic acid vectors introduced into the host cell by transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, infection, etc. In some embodiments, the choice of nucleic acid vector depends on the host cell to be used. In some embodiments, host cells are of either prokaryotic (*e.g.*, bacterial) or eukaryotic (*e.g.*, mammalian) origin.

[0190] In some embodiments, a polypeptide, for example a polypeptide construct comprising a SIRP α D1 domain variant (*e.g.*, any variant provided in Tables 2, 5, and 6) and a fusion partner such as an Fc variant are produced by culturing a host cell transformed with a nucleic acid, preferably an expression vector, containing a nucleic acid encoding the polypeptide construct (*e.g.*, Fc variant, linker, and fusion partner) under the appropriate conditions to induce or cause expression of the polypeptide construct. In some embodiments, the conditions appropriate for expression varies with the expression vector and the host cell chosen. In some embodiments, a wide variety of appropriate host cells are used, including, but not limited to, mammalian cells, bacteria, insect cells, and yeast. For example, a variety of cell lines that find use in the present disclosure are described in the ATCC[®] cell line catalog, available from the American Type Culture Collection. In some embodiments, Fc domain variants of this disclosure are expressed in a cell that is optimized not to glycosylate proteins that are expressed by such cell, either by genetic engineering of the cell line or modifications of cell culture conditions such as addition of kifunensine or by using a naturally non-glycosylating host such as a prokaryote (*E. coli*, etc.), and in some cases, modification of the glycosylation sequence in the Fc is not be needed.

Nucleic acid vector construction and host cells

[0191] A nucleic acid sequence encoding the amino acid sequence of a polypeptide of the disclosure can be prepared by a variety of methods. These methods include, but are not limited to, oligonucleotide-mediated (or site-directed) mutagenesis and PCR mutagenesis. In some embodiments, a nucleic acid molecule encoding a polypeptide of the disclosure is obtained using standard techniques, *e.g.*, gene synthesis. Alternatively, a nucleic acid molecule encoding a wild-

type SIRP α D1 domain is mutated to include specific amino acid substitutions using standard techniques, *e.g.*, QuikChangeTM mutagenesis. In some cases, nucleic acid molecules are synthesized using a nucleotide synthesizer or PCR techniques.

[0192] In some embodiments, the nucleic acids that encode a polypeptide construct, for example a polypeptide construct comprising a SIRP α D1 domain variant (*e.g.*, any variant provided in Tables 2, 5, and 6) and a fusion partner such as an Fc variant are incorporated into an expression vector in order to express the protein. A variety of expression vectors can be utilized for protein expression. Expression vectors can comprise self-replicating, extra-chromosomal vectors or vectors which integrate into a host genome. A vector can also include various components or elements. For example, in some embodiments, the vector components include, but are not limited to, transcriptional and translational regulatory sequences such as a promoter sequence, a ribosomal binding site, a signal sequence, transcriptional start and stop sequences, translational start and stop sequences, 3' and 5' untranslated regions (UTRs), and enhancer or activator sequences; an origin of replication; a selection marker gene; and the nucleic acid sequence encoding the polypeptide of interest, and a transcription termination sequence. In some embodiments, expression vectors comprise a protein operably linked with control or regulatory sequences, selectable markers, any fusion partners, additional elements, or any combinations thereof. The term "operably linked" means that the nucleic acid is placed into a functional relationship with another nucleic acid sequence. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the Fc variant, and are typically appropriate to the host cell used to express the protein. A selection gene or marker, such as, but not limited to, an antibiotic resistance gene or fluorescent protein gene, can be used to select for host cells containing the expression vector, for example by antibiotic or fluorescence expression. Various selection genes are available.

[0193] In some embodiments, the components or elements of a vector are optimized such that expression vectors are compatible with the host cell type. Expression vectors which find use in the present disclosure include, but are not limited to, those which enable protein expression in mammalian cells, bacteria, insect cells, yeast, and in in vitro systems.

[0194] In some embodiments, mammalian cells are used as host cells to produce polypeptides of the disclosure. Examples of mammalian cell types include, but are not limited to,

human embryonic kidney (HEK) (*e.g.*, HEK293, HEK 293F), Chinese hamster ovary (CHO), HeLa, COS, PC3, Vero, MC3T3, NS0, Sp2/0, VERY, BHK, MDCK, W138, BT483, Hs578T, HTB2, BT20, T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7030, and HsS78Bst cells. In some embodiments, *E. coli* cells are used as host cells to produce polypeptides of the disclosure. Examples of *E. coli* strains include, but are not limited to, *E. coli* 294 (ATCC® 31,446), *E. coli* λ 1776 (ATCC® 31,537), *E. coli* BL21 (DE3) (ATCC® BAA-1025), and *E. coli* RV308 (ATCC® 31,608).

[0195] Different host cells have characteristic and specific mechanisms for the posttranslational processing and modification of protein products (*e.g.*, glycosylation). In some embodiments, appropriate cell lines or host systems are chosen to ensure the correct modification and processing of the polypeptide expressed. Once the vectors are introduced into host cells for protein production, host cells are cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0196] In some embodiments, a polypeptide construct, for example a polypeptide construct comprising a SIRP α D1 domain variant (*e.g.*, any variant provided in Tables 2, 5, and 6) and a fusion partner such as an Fc variant are expressed in mammalian expression systems, including systems in which the expression constructs are introduced into the mammalian cells using virus such as retrovirus or adenovirus. In some embodiments, human, mouse, rat, hamster, or primate cells are utilized. Suitable cells also include known research cells, including but not limited to Jurkat T cells, NIH3T3, CHO, COS, and 293 cells. Alternately, in some embodiments, proteins are expressed in bacterial cells. Bacterial expression systems are well known in the art, and include *Escherichia coli* (*E. coli*), *Bacillus subtilis*, *Streptococcus cremoris*, and *Streptococcus lividans*. In some cases, polypeptide constructs comprising Fc domain variants are produced in insect cells such as but not limited to Sf9 and Sf21 cells or yeast cells such as but not limited to organisms from the genera *Saccharomyces*, *Pichia*, *Kluyveromyces*, *Hansenula* and *Yarrowia*. In some cases, polypeptide constructs comprising Fc domain variants are expressed *in vitro* using cell free translation systems. *In vitro* translation systems derived from both prokaryotic (*e.g.*, *E. coli*) and eukaryotic (*e.g.*, wheat germ, rabbit reticulocytes) cells are available and, in some embodiments, chosen based on the expression levels and functional properties of the protein of interest. For example, as appreciated by those skilled in the art, *in vitro* translation is required for

some display technologies, for example ribosome display. In addition, in some embodiments, the Fc domain variants are produced by chemical synthesis methods such as, but not limited to, liquid-phase peptide synthesis and solid-phase peptide synthesis. In the case of *in vitro* transcription using a non-glycosylating system such as bacterial extracts, the Fc will not be glycosylated even in presence of the natural glycosylation site and therefore inactivation of the Fc will be equivalently obtained.

[0197] In some embodiments, a polypeptide construct includes non-natural amino acids, amino acid analogues, amino acid mimetics, or any combinations thereof that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids generally refer to the 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) and pyrrolysine and selenocysteine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *e.g.*, a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, such as, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. In some embodiments, such analogs have modified R groups (such as, norleucine) or modified peptide backbones, but generally retain the same basic chemical structure as a naturally occurring amino acid.

Protein production, recovery, and purification

[0198] In some embodiments, host cells used to produce polypeptides of the disclosure are grown in media suitable for culturing of the selected host cells. Examples of suitable media for mammalian host cells include Minimal Essential Medium (MEM), Dulbecco's Modified Eagle's Medium (DMEM), Expi293™ Expression Medium, DMEM with supplemented fetal bovine serum (FBS), and RPMI-1640. Examples of suitable media for bacterial host cells include Luria broth (LB) plus necessary supplements, such as a selection agent, *e.g.*, ampicillin. In some embodiments, host cells are cultured at suitable temperatures, such as from about 20 °C to about 39 °C, *e.g.*, from about 25 °C to about 37 °C, preferably 37 °C, and CO₂ levels, such as about 5% to 10%. In some embodiments, the pH of the medium is from about pH 6.8 to pH 7.4, *e.g.*, pH 7.0, depending mainly on the host organism. If an inducible promoter is used in the expression vector, protein expression can be induced under conditions suitable for the activation of the

promoter.

[0199] In some embodiments, protein recovery involves disrupting the host cell, for example by osmotic shock, sonication, or lysis. Once the cells are disrupted, cell debris is removed by centrifugation or filtration. The proteins can then be further purified. In some embodiments, a polypeptide of the disclosure is purified by various methods of protein purification, for example, by chromatography (*e.g.*, ion exchange chromatography, affinity chromatography, and size-exclusion column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. For example, in some embodiments, the protein is isolated and purified by appropriately selecting and combining affinity columns such as Protein A column (*e.g.*, POROS Protein A chromatography) with chromatography columns (*e.g.*, POROS HS-50 cation exchange chromatography), filtration, ultra-filtration, de-salting and dialysis procedures. In some embodiments, a polypeptide is conjugated to marker sequences, such as a peptide to facilitate purification. An example of a marker amino acid sequence is a hexa-histidine peptide (His6-tag (SEQ ID NO: 223)), which can bind to a nickel-functionalized agarose affinity column with micromolar affinity. As an alternative, a hemagglutinin “HA” tag, which corresponds to an epitope derived from the influenza hemagglutinin protein can be used.

[0200] In some embodiments, polypeptides of the disclosure, for example a polypeptide construct comprising a SIRP α D1 domain variant (*e.g.*, any variant provided in Tables 2, 5, and 6) and a fusion partner such as an Fc variant are produced by the cells of a subject (*e.g.*, a human), *e.g.*, in the context of gene therapy, by administering a vector such as a viral vector (*e.g.*, a retroviral vector, adenoviral vector, poxviral vector (*e.g.*, vaccinia viral vector, such as Modified Vaccinia Ankara (MVA)), adeno-associated viral vector, and alphaviral vector) containing a nucleic acid molecule encoding a polypeptide of the disclosure. The vector, once inside a cell of the subject (*e.g.*, by transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, infection, etc.) can be used for the expression of a polypeptide disclosed herein. In some cases, the polypeptide is secreted from the cell. In some embodiments, if treatment of a disease or disorder is the desired outcome, no further action is required. In some embodiments, if collection of the protein is desired, blood is collected from the subject and the protein purified from the blood by various methods.

Methods of Treating Cancer

[0201] Provided herein are methods of treating cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) a polypeptide comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein) and (b) a chemotherapy agent (*e.g.*, at least one chemotherapy agent, such as at least two, at least three, or at least four chemotherapy agents). In some embodiments the method further comprises administering to the individual an effective amount of a therapeutic antibody (*e.g.*, at least one therapeutic antibody, such as at least two, at least three, or at least four therapeutic antibodies). Additionally or alternatively, in some embodiments the method further comprises administering to the individual an effective amount of an immunotherapeutic agent (*e.g.*, at least one immunotherapeutic agent, such as at least two, at least three, or at least four immunotherapeutic agents). Additionally or alternatively, in some embodiments, the method comprises administering the polypeptide and the chemotherapy agent in combination with one or more additional modes of therapy, including, but not limited to, *e.g.*, radiation therapy, surgery, cryoablation, and bone marrow transplant.

Combination Therapies Comprising Chemotherapy Agents, and Exemplary Chemotherapy Agents

[0202] Exemplary chemotherapy agent(s) that can be used in a method of treating cancer described herein include, without limitation, *e.g.*, methotrexate (RHEUMATREX®, Amethopterin), cyclophosphamide (CYTOXAN®), abiraterone, abemaciclib, altretamine, thalidomide (THALIDOMID®), acridine carboxamide, actimid®, actinomycin, actinomycin-D, afatinib, 17-N-allylamino-17-demethoxygeldanamycin, alectinib, alpelisib, aminopterin, amsacrine, anlotinib, anthracycline, antineoplastic, antineoplaston, apartinib, 5-azacitidine, 6-mercaptopurine, 6-thioguanine, arabinosylcytosine, axitinib, azacitidine, azathioprine, BL22, bendamustine, binimetinib, biricodar, bleomycin, bortezomib, bosutinib, brigatinib, bryostatin, busulfan, cabozantinib, calyculin, camptothecin, capecitabine, carboplatin, carmustine, ceritinib, chlorambucil, cisplatin, cladribine, clofarabine, cobimetinib, crizotinib, cytarabine, dabrafenib, dacarbazine, dacomitinib, dasatinib, daunorubicin, dexamethasone, dichloroacetic acid, discodermolide, docetaxel, doxorubicin, encorafenib, epirubicin, entrectinib, enzalutamide,

epothilone, erdafitinib, eribulin, erlotinib, estramustine, etoposide, everolimus, exatecan, exisulind, ferruginol, floxuridine, fludarabine, fluorouracil (such as 5-fluorouracil), folinic acid, fosfestrol, fotemustine, fruquintinib, ganciclovir, gefitinib, gemcitabine, gilteritinib, goserelin, hexamethylmelamine, hydroxycarbamide, hydroxyurea, IT-101, ibrutinib, icotinib, idarubicin, idelalisib, ifosfamide, imatinib, irinotectin, irinotecan, irofulven, ivosidenib, ixabepilone, laniquidar, lapatinib, larotrectinib, lenalidomide, lenvatinib, lorlatinib, lomustine, lurtotecan, mafosfamide, masoprostol, mechlorethamine, melphalan, mercaptopurine, methotrexate, methylprednisolone, mitomycin, mitotane, mitoxantrone, nelarabine, neratinib, niraparib, nilotinib, nintedanib, oblimersen, olaparib, osimertinib, oxaliplatin, nedaplatin, phenanthriplatin, picoplatin, PAC-1, paclitaxel, palbociclib, pazopanib, pemetrexed, pegfilgrastim, pentostatin, pipobroman, pixantrone, plicamycin, prednisone, ponatinib, procarbazine, proteasome inhibitors (*e.g.*, bortezomib), pyrotinib, raltitrexed, rebeccamycin, revlimid®, regorafenib, ribociclib, rubitecan, rucaparib, ruxolitinib, SN-38, salinosporamide A, satraplatin, sirolimus, sonidegib, sorafenib, streptozocin, streptozotocin, sunitinib, swainsonine, talazoparib, tariquidar, taxane, tegafur-uracil, temsirolimus, teniposide, temozolomide, testolactone, thioTEPA, tioguanine, topotecan, trabectedin, trametinib, tretinoin, trifluridine, triplatin tetranitrate, tris(2-chloroethyl)amine, troxacitabine, uracil mustard, valrubicin, vandetanib, vemurafenib, venetoclax (ABT-199), navitoclax (ABT-263), vinblastine, vincristine, vinorelbine, vismodegib, vorinostat, ziv-aflibercept (ZALTRAP®), zosuquidar, or the like.

[0203] In some embodiments, the method of treating cancer comprises administering an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRPα (*e.g.*, hSIRPα) in combination with chemotherapeutic agent(s) of a particular class. In some embodiments, the agent that blocks the interaction between CD47 and SIRPα is a polypeptide described herein (*e.g.*, a fusion polypeptide comprising a SIRPα d1 domain variant and an Fc variant; a fusion polypeptide comprising a SIRPγ variant, a SIRPβ1 variant, or a SIRPβ2 variant and an Fc variant). For example, in some embodiments, the method of treating cancer comprises administering a polypeptide (*e.g.* fusion polypeptide) described herein in combination with an adrenal inhibitor (including, but not limited to adrenal inhibitors described herein). For example, in some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an anthracycline (including, but not limited to anthracyclines described herein). In some embodiments, the method of treating cancer

comprises administering a polypeptide described herein in combination with an alkylating agent (including, but not limited to alkylating agents described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an androgen inhibitor (including, but not limited to androgen inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an antimetabolite, *e.g.*, a purine analog, (including, but not limited to antimetabolites, *e.g.*, purine analogs, described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an antitumor antibiotic (including, but not limited to antitumor antibiotics described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a BLC-2 inhibitor (including, but not limited to BLC-2 inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a BTK inhibitor (including, but not limited to BTK inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a CDK 4/6 inhibitor (including, but not limited to CDK 4/6 inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a colony stimulating factor (including, but not limited to colony stimulating factors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a corticosteroid (including, but not limited to corticosteroids described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an EGFR inhibitor (including, but not limited to EGFR inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a gonadotropin releasing hormone (GnRH) agonist (including, but not limited to GnRH agonists described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a mitotic inhibitor/microtubule inhibitor (including, but not limited to mitotic inhibitors/microtubule inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an mTOR kinase inhibitor (including, but not limited to mTOR kinase inhibitors described herein).

In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a proteasome inhibitor (including, but not limited to proteasome inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a signal transduction inhibitor, *e.g.*, a protein-tyrosine kinase inhibitor, a PAK4 inhibitor, a PI3K inhibitor, (including, but not limited to signal transduction inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a topoisomerase inhibitor, (including, but not limited to topoisomerase inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a tyrosine kinase inhibitor, (including, but not limited to tyrosine kinase inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a VEGF inhibitor, such as a VEGF1 inhibitor, a VEGF2 inhibitor, and/or a VEGF3 inhibitor (including, but not limited to VEGF inhibitors described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an agent that modulates apoptosis, *e.g.*, by modulating the activity of Bcl-2, Mcl1, Bcl-lx, etc., (including, but not limited to agents that modulate apoptosis, *e.g.*, by modulating the activity of Bcl-2, Mcl1, Bcl-lx, etc., described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a platinum-based agent, (including, but not limited to platinum-based agents described herein). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with an inhibitor of NTRK1, NTRK2, and/or NTRK3, an ALK inhibitor, a ROS inhibitor, a FLT3 inhibitor, a BRAF inhibitor, an inhibitor of MEK1 and/or MEK2, an inhibitor of HER2, HER3, and/or HER 4, an inhibitor of RET/PTC, an inhibitor of BCR-ABL, a c-KIT inhibitor, an inhibitor of PDGFR-alpha and/or PDGFR-beta, an inhibitor of FGFR1, FGFR2, FGFR3, and/or FGFR4, an Smoothened inhibitor and/or an inhibitor of PARP1, PARP2, and/or PARP3 (including, but not limited to inhibitors described herein). In some embodiments, the inhibitor is an antisense polynucleotide (such as an siRNA or an RNAi). In some embodiments, the inhibitor is a small molecule inhibitor, as described in further detail below.

[0204] In some embodiments the chemotherapeutic agent is a small molecule anti-cancer

agent (such as a small molecule inhibitor). In some embodiments, the method of treating cancer comprises administering a polypeptide described herein in combination with a small molecule inhibitor of VEGFR and/or PDGFR, a small molecule EGFR inhibitor, a small molecule ALK inhibitor, a small molecule CDK4/6 inhibitor, a small molecule PARP inhibitor, a small molecule PAK4 inhibitor, a small molecule mTOR inhibitor, a small molecule KRAS inhibitor, a small molecule TRK inhibitor, a small molecule BCL2 inhibitor, a small molecule B-raf inhibitor, a small molecule IDH inhibitor, a small molecule PI3K inhibitor, a small molecule DDR (DNA damage response) inhibitor, or a small molecule hypomethylation agent. In other cases, the targeted small molecule modulates a cellular signaling pathway of the cell expressing CD47, *e.g.*, an IDO/TDO inhibitor, AhR inhibitor, arginase inhibitor, A2a R inhibitor, TLR agonists, STING agonist, or RIG-1 agonist.

[0205] In some embodiments, the method of treating cancer comprises administering a polypeptide described herein (*e.g.*, a fusion polypeptide comprising a SIRP α d1 domain variant and an Fc variant) in combination with at least one, at least two, at least three, or at least four chemotherapeutic agents. In some embodiments where two or more chemotherapeutic agents are administered, the two or more chemotherapeutic agents are from different classes (as described above) and/or exert their anti-cancer effects via different mechanisms of action.

[0206] Further details regarding exemplary pharmaceutical compositions and preparations, exemplary dosages, and exemplary routes of administration for the fusion polypeptides described herein are provided in WO 2017/027422 and U.S. Patent No. 10,259,859, the contents of each of which are incorporated by reference in their entirety.

Combination Therapies Comprising Therapeutic Antibodies, and Exemplary Therapeutic Antibodies

[0207] In some embodiments a method of treating cancer provided herein comprises administering to the individual an effective amount of a therapeutic antibody (*e.g.*, at least one therapeutic antibody, such as at least two, at least three, or at least four therapeutic antibodies), *i.e.*, in combination with agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, a fusion polypeptide described herein) and a chemotherapeutic agent described herein (*e.g.*, at least one chemotherapeutic agent, such as at least two, at least three, or at least four chemotherapeutic agents). In some embodiments, the therapeutic antibody is conjugated to a

drug (*i.e.*, an antibody-drug conjugate, or “ADC”).

[0208] Exemplary therapeutic antibodies (*e.g.*, therapeutic monoclonal antibodies) for use in a method herein include, but are not limited to, *e.g.*, 3F8, 8H9, Abagovomab, Abciximab, Abituzumab, Abridumab, Actoxumab, Adalimumab, Adecatumumab, Aducanumab, Afelimomab, Afutuzumab, Alacizumab pegol, ALD518, Alemtuzumab, Alirocumab, Altumomab pentetate, Amatuximab, Anatumomab mafenatox, Anetumab ravtansine, Anifrolumab, Anrukinzumab (IMA-638), Apolizumab, Arcitumomab, Ascrinvacumab, Aselizumab, Atezolizumab, Atinumab, Atlizumab (tocilizumab), Atorolimumab, Avelumab, Bapineuzumab, Basiliximab, Bavixumab, Bectumomab, Begelomab, Belimumab, Benralizumab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bimekizumab, Bivatuzumab mertansine, Blinatumomab, Blosozumab, Bococizumab, Brentuximab vedotin, Briakinumab, Brodalumab, Brolucizumab, Brontictuzumab, Cabiralizumab (FPA008), Camrelizumab, Canakinumab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Capromab pendetide, Carlumab, Catumaxomab, cBR96-doxorubicin immunoconjugate, CC49, Cedelizumab, Certolizumab pegol, Cetuximab, Ch. 14.18, Citatuzumab bogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Codrituzumab, Coltuximab ravtansine, Conatumumab, Concizumab, Crenezumab, CR6261, Dacetuzumab, Daclizumab, Dalotuzumab, Dapirolizumab pegol, Daratumumab, Dectrekumab, Demcizumab, Denintuzumab mafodotin, Denosumab, Derlotuximab biotin, Detumomab, Dinutuximab, Diridavumab, Dorlimomab aritox, Drozitumab, Duligotumab, Dupilumab, Durvalumab, Dusigitumab, Ecomeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Eldelumab, Elgemtumab, Elotuzumab, Elsilimomab, Emactuzumab (RG7155), Emibetuzumab, Enavatuzumab, Enfortumab vedotin, Enlimomab pegol, Enoblituzumab, Enokizumab, Enoticumab, Ensituximab, Eptumomab cituxetan, Epratuzumab, Erlizumab, Ertumaxomab, Etaracizumab, Etrolizumab, Evinacumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab, Farletuzumab, Fasinumab, FBTA05, Felvizumab, Fezakinumab, Ficlatuzumab, Figitumumab, Firivumab, Flanvotumab, Fletikumab, Fontolizumab, Foralumab, Foravirumab, Fresolimumab, Fulranumab, Futuximab, Galiximab, Ganitumab, Gantenerumab, Gavilimomab, Gemtuzumab ozogamicin, Gevokizumab, Girentuximab, Glembatumumab vedotin, Golimumab, Gomiliximab, Guselkumab, Ibalizumab, Ibritumomab tiuxetan, Icrucumab, Idarucizumab, Igovomab, IMAB362, Imalumab, Imciromab, Imgatuzumab, Inclacumab, Indatuximab

ravtansine, Indusatumab vedotin, Infliximab, Intetumumab, Inolimomab, Inotuzumab
ozogamicin, Ipilimumab, Iratumumab, Isatuximab, Itolizumab, Ixekizumab, Keliximab,
Labetuzumab, Lambrolizumab, Lampalizumab, Lebrikizumab, Lemalesomab, Lenzilumab,
Lerdelimumab, Lexatumumab, Libivirumab, Lifestuzumab vedotin, Ligelizumab, Lilotomab
satetraxetan, Lintuzumab, Lirilumab, Lodelcizumab, Lokivetmab, Lorvotuzumab mertansine,
Lucatumumab, Lulizumab pegol, Lumiliximab, Lumretuzumab, MSB0010718C (avelumab),
Mapatumumab, Margetuximab, Maslimomab, Mavrilimumab, Matuzumab, MEDI6469,
MEDI0680, MEDI6383, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab,
Mitumomab, Mogamulizumab, Morolimumab, Motavizumab, Moxetumomab pasudotox,
Muromonab-CD3, Nacolomab tafenatox, Namilumab, Naptumomab estafenatox, Narnatumab,
Natalizumab, Nebacumab, Necitumumab, Nemolizumab, Nerelimomab, Nesvacumab,
Nimotuzumab, Nivolumab, Nofetumomab merpantan, Obiltoxaximab, Obinutuzumab,
Ocaratuzumab, Ocrelizumab, Odulimumab, Ofatumumab, Olaratumab, Olokizumab,
Omalizumab, Onartuzumab, Ontuxizumab, Opicinumab, Oportuzumab monatox, Oregovomab,
Orticumab, Otelixizumab, Otlertuzumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab,
Palivizumab, Panitumumab, Pankomab, Panobacumab, Parsatuzumab, Pascolizumab,
Pasotuxizumab, Pateclizumab, Patritumab, Pembrolizumab, Pentumomab, Perakizumab,
Pertuzumab, Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab,
Polatuzumab vedotin, Ponezumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140,
Quilizumab, Racotumomab, Radretumab, Rafivirumab, Ralpancizumab, Ramucirumab,
Ranibizumab, Raxibacumab, Refanezumab, Regavirumab, Reslizumab, Rilotumumab,
Rinucumab, Rituximab, Robatumumab, Roledumab, Romosozumab, Rontalizumab,
Rovelizumab, Ruplizumab, Sacituzumab govitecan, Samalizumab, SAR650984 (Isatuximab)
Sarilumab, Satumomab pendetide, Secukinumab, Seribantumab, Setoxaximab, Sevirumab,
Sibrotuzumab, SGN-CD19A, SGN- CD33A, Sifalimumab, Siltuximab, Simtuzumab, Sintilimab,
Siplizumab, Sirukumab, Sofituzumab vedotin, Solanezumab, Solitomab, Sonepcizumab,
Sontuzumab, Stamulumab, Sulesomab, Suvizumab, Tabalumab, Tacatuzumab tetraxetan,
Tadocizumab, Talizumab, Tanezumab, Taplitumomab paptox, Tarextumab, Tefibazumab,
Telimomab aritox, Tenatumomab, Teneliximab, Teplizumab, Teprotumumab, Tesidolumab,
TGN1412, Ticilimumab (tremelimumab), Tildrakizumab, Tigatuzumab, TNX-650, Tocilizumab
(atlizumab), Toralizumab, Toripalimab, Tosatoxumab, Tositumomab, Tovetumab,

Tralokinumab, Trastuzumab, trastuzumab-emtansine, TRBS07, Tregalizumab, Tremelimumab, Tucotuzumab celmoleukin, Tuvirumab, Ublituximab, Ulocuplumab, Urelumab, Urtoxazumab, Ustekinumab, Utomilumab (PF-05082566), Vandortuzumab vedotin, Vantictumab, Vanucizumab, Vapaliximab, Varlilumab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Visilizumab, Volociximab, Vonlerolizumab (RG7888), Vorsetuzumab mafodotin, Votumumab, Zalutumumab, Zanolimumab, Zatuximab, Ziralimumab, or Zolimomab aritox, including biosimilars of any of the preceding therapeutic antibodies.

[0209] Other exemplary therapeutic antibodies (*e.g.*, therapeutic monoclonal antibodies) that can be used in a method herein is an antibody include, but are not limited to, *e.g.*, an anti-CD20 antibody, an anti-EGFR antibody, an anti-Her2/Neu (ERBB2) antibody, an anti-EPCAM antibody, an anti-GL2 antibody, anti-GD2, anti-GD3, anti-CD2, anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-CD22, anti-CD30, anti-CD33, anti-CD39, anti-CD45, anti-CD47, anti-CD52, anti-CD56, anti-CD70, anti-CD73, anti-CD117, an anti-SIRP α antibody, an anti-LILRB1, an anti-LILRB2, an anti-LILRB4 antibody, an anti-PD-1 antibody (*e.g.*, an anti PD-1 antagonist antibody), an anti-PD-L1 antibody (*e.g.*, an anti PD-L1 antagonist antibody), an anti-PD-L2 antibody, or any antibody designed to bind to a tumor cell, a virally- or bacterially-infected cell, immune cell, or healthy normal cell, or to a cytokine, chemokine, or hormone of any kind.

[0210] In some embodiments, the therapeutic antibody used in a method herein is an antibody that binds to, *e.g.*, CS1/SLAMF7, Trop-2, VWF, vimentin, VEGFR2, VEGFR-1, VEGF, VEGF-A, TYRP1 (glycoprotein 75), TWEAK receptor, tumor specific glycosylation of MUC1, tumor antigen CTAA16.88, TRAIL-R2, TRAIL-R1, TNF-alpha, TGF-beta, TGF beta 2, TGF beta 1, TFPI, tenascin C, TEM1, TAG-72, T-cell receptor, STEAP1, sphingosine-1-phosphate, SOST, SLAMF7, BCL-2, selectin P, SDC1, sclerostin, RTN4, RON, Rhesus factor, RHD, respiratory syncytial virus, RANKL, rabies virus glycoprotein, platelet-derived growth factor receptor beta, phosphatidylserine, phosphate-sodium co-transporter, PDGF-R alpha, PDCD1, PD-1, PD-L1, PCSK9, oxLDL, OX-40, NRPI, Notch receptor 4, Notch receptor 3, Notch receptor 2, Notch receptor 1, NOGO-A, NGF, neural apoptosis-regulated proteinase 1, NCA-90 (granulocyte antigen), NARP-1, N-glycolylneuraminic acid, myostatin, myelin-associated glycoprotein, mucin CanAg, MUC1, MSLN, MS4A1, MIF, mesothelin, MCP-1, LTA, LOXL2, lipoteichoic acid, LINGO-1, LFA-1 (CD11a), Lewis-Y antigen, L-selectin (CD62L), KIR2D, ITGB2 (CD18), ITGA2, interferon alpha/beta receptor, interferon receptor,

interferon gamma-induced protein, integrin $\alpha v\beta 3$, integrin $\alpha II\beta 3$, integrin $\alpha 7\beta 7$, integrin $\alpha 5\beta 1$, integrin $\alpha 4\beta 7$, integrin $\alpha 4$, insulin-like growth factor I receptor, Influenza A hemagglutinin, ILGF2, IL9, IL6, IL4, IL3 IRA, IL23, IL1 7A, IL-6 receptor, IL-6, IL-S, IL-4, IL-23, IL-22, IL- I , IL- I 7A, IL-I 7, IL-13, IL- I 2, IL- I, IL 20, IGHE, IgG4, IGF-I, IGF- I receptor, IgE Fc region, IFN-gamma, IFN-alpha, ICAM-1 (CD54), human TNF, human scatter factor receptor kinase, Hsp90, HNGF, HLA-DR, HIV-1, histone complex, HHGFR, HGF, HER3, HER2, HER2/neu, HER1, hepatitis B surface antigen, hemagglutinin, GUCY2C, GPNMB, GMCSF receptor alpha-chain, glypican 3, GD3 ganglioside, GD2, ganglioside GD2, Frizzled receptor, folate receptor 1, folate hydrolase, fibronectin extra domain-B, fibrin II, beta chain, FAP, F protein of respiratory syncytial virus, ERBB3, episialin, EpCAM, endotoxin, EGFR, EGFL7, E. coli shiga toxin type-2, E. coli shiga toxin type- I, DRS, DPP4, DLL4, dabigatran, cytomegalovirus glycoprotein B, CTLA-4, CSF2, CSF1R, clumping factor A, CLDN18.2, ch4DS, CFD, CEA-related antigen, CEA, CD80, CD79B, CD74, CD73, CD70, CD6, CD56, CD52, CD51, CD5, CD44 v6, CD41, CD40 ligand, CD40, CD4, CD39, CD38, CD37, CD33, CD30 (TNFRSF8), CD123, CD138, CD3 epsilon, CD3, CD28, CD274, CD27, CD2S (a chain of IL-2 receptor), CD23 (IgE receptor), CD221, CD22, CD200, CD20, CD2, CD19, CD137, CD154, CD152, CD15, CD147 (basigin), CD140a, CD125, CD11, CD-18, CCR5, CCR4, CCL11 (eotaxin-I), cardiac myosin, carbonic anhydrase 9 (CA-IX), Canis lupus familiaris IL31, CA-125, C5, C242 antigen, C-X-C chemokine receptor type 4, beta-amyloid, BAFF, B7-H3, B-lymphoma cell, AOC3 (VAP-I), anthrax toxin, protective antigen, angiopoietin 3, angiopoietin 2, alpha-fetoprotein, AGS-22M6, adenocarcinoma antigen, ACVR2B, activin receptor-like kinase I, 5T4, 5AC, 4- IBB or 1-40-beta-amyloid.

[0211] In some embodiments, the therapeutic antibody used in a method herein binds to an antigen expressed by a cancer cell (*e.g.*, expressed on the surface of a cancer cell). Exemplary antigens expressed by cancers are known in the art and include, without limitation, *e.g.*, CD19, CD20, CD22, CD30, CD33, CD38, CD52, CD56, CD70, CD74, CD79b, CD123, CD138, CS1/SLAMF7, Trop-2, 5T4, BCMA, Mucin 1, Mucin 16, PTK7, PD-L1, STEAP1, Endothelin B Receptor, mesothelin, EGFRvIII, ENPP3, SLC44A4, GNMB, nectin 4, NaPi2b, LIV-1A, Guanylyl cyclase C, DLL3, EGFR, HER2, VEGF, VEGFR, integrin $\alpha V\beta 3$, integrin $\alpha 5\beta 1$, MET, IGF1R, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, Le^y, EpCAM, CEA, gpA33, PSMA, TAG72, a mucin, CAIX, EPHA3, folate receptor α , GD2, GD3, and an MHC/peptide complex

comprising a peptide from NY-ESO-1/LAGE, SSX-2, a MAGE family protein, MAGE-A3, gp100/pmel17, Melan-A/MART1, gp75/TRP1, tyrosinase, TRP2, CEA, PSA, TAG-72, immature laminin receptor, MOK/RAGE-1, WT-1, SAP-1, BING-4, EpCAM, MUC1, PRAME, survivin, BRCA1, BRCA2, CDK4, CML66, MART-2, p53, Ras, β -catenin, TGF- β RII, HPV E6, or HPV E7. For example, in some embodiments, a polypeptide described herein is administered in combination with a chemotherapeutic agent (*e.g.*, at least one chemotherapeutic agent) and a monoclonal antibody that binds CD123 (also known as IL-3 receptor alpha), such as talacotuzumab (also known as CSL362 and JNJ-56022473).

[0212] In some embodiments, the therapeutic antibody (*e.g.*, therapeutic monoclonal antibody) used in a method herein is an antibody that binds an antigen expressed by an NK cell. Exemplary antigens expressed by an NK cell include, without limitation, NKR-P1A (KLRB1), CD94 (NKG2A), KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DS1, CD94 (NKG2C/E), NKG2D, CD160 (BY55), CD16 (Fc γ RIIIA), NKp46 (NCR1), NKp30 (NCR3), NKp44 (NCR2), DNAM1 (CD226), CRTAM, CD27, NTB-A (SLAMF6), PSGL1, CD96 (Tactile), CD100 (SEMA4D), NKp80 (KLRF1, CLEC5C), SLAMF7 (CRACC, CS1, CD319), and CD244 (2B4, SLAMF4).

Combination Therapies Comprising Immunotherapeutic Agents, and Exemplary Immunotherapeutic Agents

[0213] In some embodiments a method of treating cancer provided herein comprises administering to the individual an effective amount of an immunotherapeutic agent (*e.g.*, at least one immunotherapeutic agent, such as at least two, at least three, or at least four immunotherapeutic agents), *i.e.*, in combination with an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, a polypeptide described herein) and a chemotherapeutic agent described herein (*e.g.*, at least one chemotherapeutic agent, such as at least two, at least three, or at least four chemotherapeutic agents).

[0214] In some embodiments, an immunotherapeutic agent refers to any therapeutic that targets the immune system and promotes a therapeutic redirection of the immune system, such as a modulator of a costimulatory pathway, cancer vaccine, recombinantly modified immune cell, etc. Exemplary and non-limiting immunotherapeutic agents are described *infra*. In some embodiments, the immunotherapeutic agent is or comprises an antibody. Exemplary targets of

immunotherapeutic antibodies are known in the art and include, without limitation, BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, Siglec-3, Siglec-7, Siglec-9, Siglec-10, Siglec-15, FGL-1, CD200, CD200R, CSF-1R, CD24, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, arginase, IDO, TDO, AhR, EP2, COX-2, CCR2, CCR-7, CXCR1, CX3CR1, CXCR2, CXCR3, CXCR4, CXCR7, TGF- β RI, TGF- β RII, c-Kit, CD244, L-selectin/CD62L, CD11b, CD11c, CD68, 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, KIRg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, TNFR2, PVRIG, DNAM, and ICOS.

[0215] Immunotherapeutic agents that are approved or in late-stage clinical testing include, without limitation, ipilimumab, pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and the like. In certain embodiments, the agent that blocks the interaction between CD47 and SIRP α (such as a polypeptide described herein) is administered in combination with an inhibitor of the PD-L1/PD-1 pathway, *e.g.*, an antibody, a small molecule, or polypeptide that blocks the interaction between PD-L1 and PD-1 (*e.g.*, by binding to PD-1 or PD-L1). In some embodiments, the inhibitor of the PD-L1/PD-1 pathway is an antisense polynucleotide. In some embodiments, the inhibitor of the PD-L1/PD-1 pathway is an anti-PD-L1 or anti-PD-1 antagonist antibody (*e.g.*, an anti-PD-1 or anti-PD-L1 antagonist antibody described elsewhere herein). As demonstrated herein, combined administration of an agent that blocks the interaction between CD47 and SIRP α (such as a polypeptide described herein) and an inhibitor of the PD-L1/PD-1 pathway can result in synergistic anti-tumor activity. In some embodiments, the immunotherapeutic agent is or comprises a vaccine, oncolytic virus, adoptive cell therapy, cytokine, or small molecule immunotherapeutic agent. Examples of such immunotherapeutic agents are known in the art. For example, adoptive cell therapies and therapeutics can include without limitation chimeric antigen receptor T-cell therapy (CAR-T), tumor infiltrating lymphocytes (TILs), TCR engineered T cells, TCR engineered NK cell, and macrophage cell products. Vaccines can include without limitation polynucleotide vaccines, polypeptide vaccines, or cell-based (*e.g.*, tumor or dendritic cell-based) vaccines. Various cytokines useful for the treatment of cancer are known and include without limitation IL-2, IL-15, IL-7, IL-10, IL-12, IL21, TNF α , IFNs, GM-CSF, and engineered cytokine mutants. Small molecule immunotherapeutic agents can include without limitation IDO/TDO inhibitors, AhR inhibitors, arginase inhibitors, A2a R inhibitors, TLR agonists, STING agonists, and RIG-1 agonists.

[0216] In some embodiments where the agent that blocks the interaction between CD47 and SIRP α (such as a polypeptide described herein) and the chemotherapeutic agent (*e.g.*, at least one chemotherapeutic agent) are administered in combination with further agent(s) described herein (*e.g.*, therapeutic antibodies, small molecule inhibitors, immunotherapeutic agents, etc.), the further agent(s) are from different classes and/or exert their anti-cancer effects via different mechanisms of action. For example, in some embodiments, the method of treating cancer comprises administering an agent that blocks the interaction between CD47 and SIRP α (such as a polypeptide described herein) in combination with a chemotherapeutic agent (including, but not limited to those described herein) and a therapeutic antibody (including, but not limited to those described herein, *e.g.*, an anti-HER2 antibody). In some embodiments, the agent that blocks the interaction between CD47 and SIRP α (such as a polypeptide described herein) is administered in combination with a chemotherapeutic agent (including, but not limited to those described herein) and a small molecule inhibitor (including, but not limited to those described herein). Other combinations are also contemplated.

[0217] In some embodiments, the agent that blocks the interaction between CD47 and SIRP α (such as a polypeptide described herein) is administered in combination with one or more agents including, without limitation, *e.g.*, anti-diarrheal agents, anti-emetic agents, analgesics, opioids and/or non-steroidal anti-inflammatory agent.

Combination Therapies That Comprise Additional Mode(s) of Therapy

[0218] In some embodiments, the agent that blocks the interaction between CD47 and SIRP α (such as a polypeptide described herein) is administered in combination with at least one chemotherapy agent and one or more additional modes of therapy. In some embodiments, the one or more additional modes therapy comprises radiotherapy (*e.g.*, gamma-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells, microwaves, UV radiation, or gene therapy. For example, therapeutic genes for gene therapy include, but are not limited to, an antisense version of an inducer of cellular proliferation (oncogene), an inhibitor of cellular proliferation (tumor suppressor), or an inducer of programmed cell death (pro-apoptotic gene). In some embodiments, any one or more of the combination therapies described herein are administered in conjunction with a surgery (*e.g.*, resection).

Exemplary Therapeutic Combinations

[0219] In some embodiments, the method of treating cancer comprises administering to an individual in need thereof an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) in combination with trastuzumab and one or agents selected from: lenalidomide, ibrutinib, palbociclib, enzalutamide, pemetrexed, nilotinib, abiraterone, imatinib, palbociclib, erlotinib, bortezomib, enzalutamide, cyclophosphamide, carboplatin, cisplatin, oxaliplatin, 5-fluorouracil, 6-mercaptopurine, cytarabine, gemcitabine, methotrexate, bleomycin, daunorubicin, doxorubicin, docetaxel, estramustine, paclitaxel, vinblastine, etoposide, irinotecan, teniposide, topotecan, prednisone, methylprednisolone, and dexamethasone. In some embodiments, the agent that blocks the interaction between CD47 and SIRP α is a polypeptide described herein (*e.g.*, a fusion polypeptide comprising a SIRP α D1 domain variant and an Fc variant).

[0220] In some embodiments, the method of treating cancer comprises administering an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) in combination with trastuzumab, ramucirumab, and paclitaxel. In some embodiments, the agent that blocks the interaction between CD47 and SIRP α is a polypeptide described herein (*e.g.*, a fusion polypeptide comprising a SIRP α D1 domain variant and an Fc variant).

[0221] In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual an effective amount of: (a) a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant, (b) an anti-HER2 antibody, (c) an anti-VEGF2 antibody, and (d) paclitaxel; wherein the SIRP α D1 domain variant comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85; wherein the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat, wherein the cancer is gastric cancer or gastroesophageal junction (GEJ) cancer, and wherein the individual has received at least one

prior therapy for the gastric or the GEJ cancer. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a dimer (*e.g.*, homodimer). In some embodiments, the gastric cancer or GEJ cancer is a HER2-overexpressing (*e.g.*, HER2⁺) gastric cancer or a HER2-overexpressing GEJ cancer. In some embodiments, the individual has received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, or with an anti HER2 antibody and a platinum-based chemotherapy agent. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with trastuzumab and fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil). In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with trastuzumab and platinum-containing chemotherapy. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with trastuzumab, fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil), and platinum-containing chemotherapy. In some embodiments, the individual has not received prior therapy with an anti-CD47 agent or an anti-SIRP α agent. In some embodiments, the anti-HER2 antibody is trastuzumab. In some embodiments, the anti-VEGF antibody is ramucirumab. In some embodiments, the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered to the individual at a dosage and frequency described below. In some embodiments, the anti-HER2 antibody (*e.g.*, trastuzumab) is administered to the individual at a dosage and frequency described below. In some embodiments the anti-HER2 antibody (*e.g.*, trastuzumab) is administered to the individual according to the dosage and frequency indicated in the prescribing label. For example, for the United States, details regarding the dosage and frequency of administration for trastuzumab can be found at [www\(dot\)accessdata\(dot\)fda\(dot\)gov/drugsatfda_docs/label/2010/103792s5250lbl\(dot\)pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/103792s5250lbl.pdf).

[0222] In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of about 10 to about 60 mg/kg once a week (qw). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of about 10 to about 60 mg/kg once every two weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the

Fc domain variant is administered at a dose of 10 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 15 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 20 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 30 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 40 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 50 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 60 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 10 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 15 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 20 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 30 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 40 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 50 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 60 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant comprises a SIRP α variant that comprises SEQ ID NO: 81 or SEQ ID NO: 85 and an the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is

according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a dimer (*e.g.*, homodimer). In some embodiments, the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered to the individual in combination with (a) an anti-HER2 antibody, (b) an anti-VEGF2 antibody, and (c) paclitaxel. In some embodiments, the cancer is gastric cancer or gastroesophageal junction (GEJ) cancer. In some embodiments, the individual has received at least one prior therapy for the gastric or the GEJ cancer. In some embodiments, the gastric cancer or GEJ cancer is a HER2-overexpressing (*e.g.*, HER2⁺) gastric cancer or a HER2-overexpressing GEJ cancer. In some embodiments, the individual has received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, or with an anti-HER2 antibody and a platinum-based chemotherapy agent. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab) and fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil). In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab) and platinum-containing chemotherapy. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab), fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil), and platinum-containing chemotherapy. In some embodiments, the individual has not received prior therapy with an anti-CD47 agent or an anti-SIRP α agent. In some embodiments, the anti-HER2 antibody is trastuzumab. In some embodiments, the anti-VEGF antibody is ramucirumab. In some embodiments the anti-VEGFR-2 antibody (*e.g.*, ramucirumab) is administered to the individual according to the dosage and frequency indicated in the prescribing label. For example, for the United States, details regarding the dosage and frequency of administration for ramucirumab can be found at [www\(dot\)accessdata\(dot\)fda\(dot\)gov/drugsatfda_docs/label/2020/125477s034lbl\(dot\)pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125477s034lbl.pdf). In some embodiments, the anti-HER2 antibody (*e.g.*, trastuzumab) is administered to the individual at a dosage and frequency described below. In some embodiments the anti-HER2 antibody (*e.g.*,

trastuzumab) is administered to the individual according to the dosage and frequency indicated in the prescribing label. For example, for the United States, details regarding the dosage and frequency of administration for trastuzumab can be found at [www\(accessdata\(dot\)fda\(dot\)gov/drugsatfda_docs/label/2010/103792s5250lbl\(dot\)pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/103792s5250lbl.pdf).

[0223] In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRPα D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks (*e.g.*, an initial dose of 8 mg/kg during week 1 followed by a 6 mg/kg dose given during week two, and a 6 mg/kg dose once every three weeks after the first 6 mg/kg dose). In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRPα D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks and ramucirumab at a dose of 8 mg/kg once every other week (*e.g.*, on Days 1 and 15 during each 28 day cycle). In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRPα D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks, ramucirumab at a dose of 8 mg/kg once every other week (*e.g.*, on Days 1 and 15 during each 28 day cycle), and paclitaxel at a dose of 80 mg/m² once a week for three weeks during every 4 week cycle (*e.g.*, on Days 1, 8, and 15 during every 28 day cycle). In some embodiments, the polypeptide comprising a SIRPα D1 domain variant and an Fc domain variant comprises a SIRPα variant that comprises SEQ ID NO: 81 or SEQ ID NO: 85 and an the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises the

amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a dimer (*e.g.*, homodimer). In some embodiments, the cancer is gastric cancer or gastroesophageal junction (GEJ) cancer. In some embodiments, the individual has received at least one prior therapy for the gastric or the GEJ cancer. In some embodiments, the gastric cancer or GEJ cancer is a HER2-overexpressing (*e.g.*, HER2⁺) gastric cancer or a HER2-overexpressing GEJ cancer. In some embodiments, the individual has received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, or with an anti HER2 antibody and a platinum-based chemotherapy agent. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab) and fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil). In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab) and platinum-containing chemotherapy. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab), fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil), and platinum-containing chemotherapy. In some embodiments, the individual has not received prior therapy with an anti-CD47 agent or an anti-SIRP α agent.

[0224] In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks (*e.g.*, an initial dose of 8 mg/kg during week 1 followed by a 6 mg/kg dose given during week two, and a 6 mg/kg dose once every three weeks after the first 6 mg/kg dose). In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks and ramucirumab at a dose of 8 mg/kg once every other week (*e.g.*, on Days 1 and 15 during each 28 day cycle). In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an

initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks, ramucirumab at a dose of 8 mg/kg once every other week (*e.g.*, on Days 1 and 15 during each 28 day cycle), and paclitaxel at a dose of 80 mg/m² once a week for three weeks during every 4 week cycle (*e.g.*, on Days 1, 8, and 15 during every 28 day cycle). In some embodiments, the polypeptide comprising a SIRPα D1 domain variant and an Fc domain variant comprises a SIRPα variant that comprises SEQ ID NO: 81 or SEQ ID NO: 85 and an the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a dimer (*e.g.*, homodimer). In some embodiments, the cancer is gastric cancer or gastroesophageal junction (GEJ) cancer. In some embodiments, the individual has received at least one prior therapy for the gastric or the GEJ cancer. In some embodiments, the gastric cancer or GEJ cancer is a HER2-overexpressing (*e.g.*, HER2⁺) gastric cancer or a HER2-overexpressing GEJ cancer. In some embodiments, the individual has received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, or with an anti HER2 antibody and a platinum-based chemotherapy agent. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy an anti-HER2 antibody (*e.g.*, trastuzumab) and fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil). In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab) and platinum-containing chemotherapy. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab), fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil), and platinum-containing chemotherapy. In some embodiments, the individual has not received prior therapy with an anti-CD47 agent or an anti-SIRPα agent.

[0225] In some embodiments, provided is a method of treating cancer in an individual,

comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, an initial dose of 6 mg/kg, followed by a 4 mg/kg dose two weeks after the initial 6 mg/kg dose, followed by a 4 mg/kg dose every two weeks after the first 4 mg/kg dose). In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, as described elsewhere herein) and ramucirumab at a dose of 8 mg/kg once every two weeks (*e.g.*, on Days 1 and 15 of every 28 day cycle). In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, as described elsewhere herein) and ramucirumab at a dose of 8 mg/kg once every two weeks (*e.g.*, on Days 1 and 15 of every 28 day cycle), and paclitaxel at a dose of 80 mg/m² once a week for three weeks of every 4 week cycle (*e.g.*, on Days 1, 8 and 15 of a 28-day cycle). In some embodiments, the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant comprises a SIRP α variant that comprises SEQ ID NO: 81 or SEQ ID NO: 85 and an the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a dimer (*e.g.*, homodimer). In some embodiments, the cancer is gastric cancer or gastroesophageal junction (GEJ) cancer. In some embodiments, the individual has received at least one prior therapy for the gastric or the GEJ cancer. In some embodiments, the

gastric cancer or GEJ cancer is a HER2-overexpressing (*e.g.*, HER2⁺) gastric cancer or a HER2-overexpressing GEJ cancer. In some embodiments, the individual has received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, or with an anti-HER2 antibody and a platinum-based chemotherapy agent. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy an anti-HER2 antibody (*e.g.*, trastuzumab) and fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil). In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab) and platinum-containing chemotherapy. In some embodiments, the individual progressed (*e.g.*, experienced disease progression) during or after prior therapy with an anti-HER2 antibody (*e.g.*, trastuzumab), fluoropyrimidine-containing chemotherapy (*e.g.*, fluorouracil), and platinum-containing chemotherapy. In some embodiments, the individual has not received prior therapy with an anti-CD47 agent or an anti-SIRPα agent.

Exemplary Cancers

[0226] In some embodiments, the cancer treated by a method provided herein is breast cancer, lung cancer, adenocarcinoma of the lung, squamous cell lung cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), head and neck cancer, mesothelioma, brain cancer, brain tumor, abdominal cancer, colon cancer, colorectal cancer, esophageal cancer, parapharyngeal cancer, gastrointestinal cancer, glioma, liver cancer, gastric cancer, oral cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, renal cancer, urinary bladder cancer, urinary tract cancer, pancreatic cancer, retinoblastoma, cervical cancer, uterine cancer, Wilm's tumor, multiple myeloma, skin cancer, lymphoma, leukemia, blood cancer, thyroid cancer, bone cancer, adenocystic tumor, chondrosarcoma, pancreatic islet cell tumor, neuroendocrine tumor, prostate cancer, glioblastoma, endometrial carcinoma, endometrial cancer, leiomyosarcoma, gall bladder cancer, hepatocellular cancer, a melanoma, or a solid tumor.

[0227] In some embodiments, the cancer treated by a method provided herein is gastric cancer. In some embodiments, the cancer treated by a method provided herein is gastric adenocarcinoma. In some embodiments, the cancer treated by a method provided herein is gastroesophageal junction adenocarcinoma. In some embodiments, the cancer treated by a

method provided herein is HER2-overexpressing gastroesophageal junction adenocarcinoma.

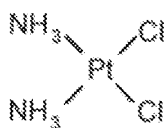
Methods of Treating Solid Tumor

[0228] In some embodiments, provided is a method of treating solid tumor in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) and (b) a platinum-based chemotherapy agent. In some embodiments, the solid tumor is colon cancer (*e.g.*, colon carcinoma), lung cancer, head and neck cancer, esophageal cancer, breast cancer, bladder cancer, ovarian cancer, cervical cancer, testicular cancer, brain tumor, mesothelioma, or neuroblastoma. In some embodiments, the platinum-based chemotherapy agent is carboplatin, cisplatin, oxaliplatin, nedaplatin, triplatin tetranitrate, phenanthriplatin, picoplatin, and/or satraplatin. In some embodiments, the platinum-based chemotherapy agent is cisplatin. In some embodiments, the agent is a polypeptide (*e.g.*, fusion polypeptide) comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises a SIRP α D1 domain variant that comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) administered to the individual comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a homodimer. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) and the platinum-based chemotherapy agent (*e.g.*, cisplatin) are administered simultaneously, concurrently, or sequentially.

[0229] Platinum agents (such as carboplatin, cisplatin, oxaliplatin, nedaplatin, triplatin tetranitrate, phenanthriplatin, picoplatin, and satraplatin) are widely used antitumor drugs that

cause crosslinking of DNA as monoadduct, interstrand crosslinks, intrastrand crosslinks or DNA protein crosslinks. Platinum agents typically act on the adjacent N-7 position of guanine, forming a 1, 2 intrastrand crosslink (*Poklar et al. (1996). Proc. Natl. Acad. Sci. U.S.A. 93 (15): 7606–11; Rudd et al. (1995). Cancer Chemother. Pharmacol. 35 (4): 323–6*). The resultant crosslinking inhibits DNA repair and/or DNA synthesis in cancer cells.

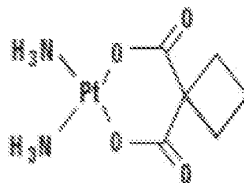
[0230] Cisplatin is an exemplary platinum coordination compound used in the methods described herein. The chemical name for cisplatin is dichloroplatinum diammoniate, and cisplatin has the following structural formula:



[0231] Cisplatin is an inorganic and water-soluble platinum complex with the molecular formula of $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ and a molecular weight of 300.046. After undergoing hydrolysis, it reacts with DNA to produce both intra and interstrand crosslinks. These crosslinks appear to impair replication and transcription of DNA. The cytotoxicity of cisplatin correlates with cellular arrest in the G2 phase of the cell cycle. Cisplatin, which has been assigned the CAS Registry No. 15663-27-1, is commercially available as PLATINOL®, PLATINOL®-AQ, CDDP, CISPLAN, CISPLAT, PLATIKEM, PLATIONCO, PRACTICIS, PLATICIS, BLASTOLEM, CISMAL, CISPLAN, CISPLATINUM, CISTEEN, DUPLAT, KEMOPLAT, ONCOPLATIN-AQ, PLATINEX, PLATIN, TEVAPLATIN, and others. Complete information about cisplatin preparation, dispensing, dosage, and administration schedule can be found in local package insert (for the United States, see, e.g., [www\(dot\)accessdata\(dot\)fda\(dot\)gov/drugsatfda_docs/label/2011/018057s080lbl\(dot\)pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/018057s080lbl.pdf) and [www\(dot\)accessdata\(dot\)fda\(dot\)gov/drugsatfda_docs/label/2015/018057s083lbl\(dot\)pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/018057s083lbl(dot)pdf)). In some embodiments, the cisplatin is administered in accordance with the dosing and frequency recommended in the local package insert.

[0232] Carboplatin is another exemplary platinum coordination compound used in the methods described herein. The chemical name for carboplatin is platinum, diammine [1,1cyclobutane-dicarboxylato(2-)-0,0]-, (SP-4-2), and carboplatin has the following structural

formula:



[0233] Carboplatin is a water-soluble platinum complex with the molecular formula of $C_6H_{12}N_2O_4Pt$ and a molecular weight of 373.26. Carboplatin has been assigned the CAS Registration Number 41575-94-4, and its mechanism of action is similar to that of cisplatin. Carboplatin is typically prescribed more commonly than cisplatin. Carboplatin is commercially available as PARAPLATIN®, BLASTOCARB®, BLASTOPLATIN®, CARBOKEM®, CARBOMAX®, PARAPLATIN®, CARBOPA®, KARPLAT®, and others. Complete information about carboplatin preparation, dispensing, dosage, and administration schedule can be found in local package insert (for the United States, see, *e.g.*, [www\(dot\)accessdata\(dot\)fda\(dot\)gov/drugsatfda_docs/label/2010/020452s005lbl\(dot\)pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020452s005lbl.pdf) and [www\(dot\)accessdata.fda\(dot\)gov/drugsatfda_docs/label/2012/077139Orig1s016lbl\(dot\)pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/077139Orig1s016lbl.pdf)). In some embodiments, the carboplatin is administered in accordance with the dosing and frequency recommended in the local package insert.

[0234] In some embodiments, provided is a method of treating solid tumor in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRPα (*e.g.*, hSIRPα), (b) an anti-HER 2 antibody, and (c) an anti-PD-L1 antibody. In some embodiments the anti-HER2 antibody is trastuzumab (CAS Registry No. 180288-69-1). In some embodiments the anti-PD-L1 antibody is atezolizumab (CAS Registry No. 1380723-44-3), avelumab (CAS Registry No. 1537032-82-8), or durvalumab (CAS Registry No. 1428935-60-7). In some embodiments, the agent is a polypeptide (*e.g.*, fusion polypeptide) comprising a SIRPα D1 domain variant (*e.g.*, a SIRPα D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises a SIRPα D1 domain variant that comprises the amino acid

sequence of SEQ ID NO: 81 or SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) administered to the individual comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a homodimer. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide), the anti-HER2 antibody, the anti-PD-L1 antibody (*e.g.*, an anti PD-L1 antagonist antibody) are administered simultaneously, concurrently, or sequentially. In some embodiments, the solid tumor is colon cancer, lung cancer, head and neck cancer, esophageal cancer, breast cancer, bladder cancer, ovarian cancer, cervical cancer, testicular cancer, endometrial cancer, liver cancer, gastric cancer, gastroesophageal junction cancer, brain tumor, mesothelioma, or neuroblastoma. In some embodiments, the solid tumor is HER2⁺ solid tumor. In some embodiments, the solid tumor is colon cancer (*e.g.*, HER2⁺ colon cancer).

Methods of Treating Gastric Cancer (GC) or Gastroesophageal Junction (GEJ) Cancer

[0235] In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRPα (*e.g.*, hSIRPα), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel. In some embodiments the anti-HER2 antibody is trastuzumab (CAS Registry No. 180288-69-1). In some embodiments the anti-VEGFR2 antibody is ramucirumab (CAS Registry No. 947687-13-0). In some embodiments, the agent is a polypeptide (*e.g.*, fusion polypeptide) comprising a SIRPα D1 domain variant (*e.g.*, a SIRPα D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises a SIRPα D1 domain variant that comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A,

L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) administered to the individual comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a dimer (*e.g.*, homodimer). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide), the anti-HER2 antibody, the anti-VEGFR2 antibody, and the paclitaxel are administered simultaneously, concurrently, or sequentially. In some embodiments, the polypeptide (*e.g.* fusion polypeptide) is administered to the individual at a dose of 10 mg/kg once a week or 15 mg/kg once a week or 30 mg/kg once every two weeks. In some embodiments, the individual receiving treatment has gastric or GEJ adenocarcinoma. In some embodiments, the individual receiving treatment has HER2⁺ gastric cancer or HER2⁺ GEJ cancer (*e.g.*, a HER2-overexpressing gastric or GEJ cancer). In some embodiments, the HER2⁺ gastric cancer or HER2⁺ GEJ cancer is advanced and/or metastatic. In some embodiments, the individual receiving treatment has gastric or GEJ cancer that has progressed during or after prior treatment(s) comprising anti-HER2 antibody (*e.g.*, trastuzumab). In some embodiments, the individual receiving treatment has gastric or GEJ cancer that has progressed during or after prior treatment(s) comprising anti-HER2 antibody (*e.g.*, trastuzumab) and a fluoropyrimidine (*e.g.*, fluorouracil, also known as 5-fluorouracil). In some embodiments, the individual receiving treatment has gastric or GEJ cancer that has progressed during or after prior treatment(s) comprising a fluoropyrimidine (*e.g.*, fluorouracil). In some embodiments, the individual receiving treatment has gastric or GEJ cancer that has progressed during or after prior treatment(s) comprising a platinum-based chemotherapeutic agent (*e.g.*, carboplatin or cisplatin). In some embodiments, the individual receiving treatment has gastric or GEJ cancer that has progressed during or after prior treatments(s) comprising anti-HER2 antibody (*e.g.*, trastuzumab) and a platinum-based chemotherapeutic agent (*e.g.*, carboplatin or cisplatin). In some embodiments, the individual receiving treatment has gastric or GEJ cancer (*e.g.*, HER2⁺ gastric cancer or GEJ cancer) that has progressed during or after prior treatment(s) comprising anti-

HER2 antibody (*e.g.*, trastuzumab) and/or a fluoropyrimidine (*e.g.*, fluorouracil), and/or a platinum-based chemotherapeutic agent (*e.g.*, carboplatin or cisplatin). In some embodiments, the individual receiving treatment has gastric or GEJ cancer (*e.g.*, HER2⁺ gastric cancer or GEJ cancer) that has progressed during or after prior treatment(s) comprising anti-HER2 antibody (*e.g.*, trastuzumab) and/or a fluoropyrimidine (*e.g.*, fluorouracil). In some embodiments, the individual receiving treatment has gastric or GEJ cancer (*e.g.*, HER2⁺ gastric cancer or GEJ cancer) that has progressed during or after prior treatment(s) with a platinum-based chemotherapeutic agent (*e.g.*, carboplatin or cisplatin). In some embodiments, the individual failed (*e.g.*, relapsed after or did not respond to) prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine (*e.g.*, fluorouracil), with an anti-HER2 antibody and a platinum-based chemotherapy agent (*e.g.*, carboplatin or cisplatin), or with a platinum-based chemotherapy agent (*e.g.*, carboplatin or cisplatin). In some embodiments, the fluoropyrimidine was fluorouracil (also known as 5-fluorouracil). In some embodiments, the individual receiving treatment has undergone a prior anti-VEGFR2 antibody-containing therapy for gastric or GEJ cancer. In some embodiments, the prior anti-VEGFR2 antibody-containing therapy was a therapy comprising ramucirumab. In some embodiments, the individual receiving treatment has not undergone a prior anti-VEGFR2 antibody-containing therapy (*e.g.*, therapy comprising ramucirumab) for gastric or GEJ cancer. In some embodiments, the individual receiving treatment has undergone at least one, at least two, at least three, or at least four prior therapies for gastric or GEJ cancer. In some embodiments, treatment with the polypeptide, the anti-HER2 antibody, the anti-VEGFR2 antibody, and the paclitaxel does not result in adverse effects. In some embodiments, treatment with the polypeptide, the anti-HER2 antibody, the anti-VEGFR2 antibody, and the paclitaxel results in only low grade adverse effects.

[0236] In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRPα (*e.g.*, hSIRPα), (b) an anti-PD-1 antibody (*e.g.*, an anti-PD-1 antagonist antibody), (c) an anti-HER2 antibody, (d) 5-fluorouracil and (e) a platinum-based chemotherapeutic agent. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that

blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-PD-1 antibody (*e.g.*, an anti-PD-1 antagonist antibody), (c) an anti-HER2 antibody, (d) capecitabine, and (e) a platinum-based chemotherapeutic agent. In some embodiments the anti-PD-1 antibody is pembrolizumab (CAS Registry No. 1374853-91-4). In some embodiments the anti-HER2 antibody is trastuzumab (CAS Registry No. 180288-69-1). In some embodiments, the platinum-based chemotherapeutic agent is cisplatin. In some embodiments, the agent is a polypeptide (*e.g.*, fusion polypeptide) comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises a SIRP α D1 domain variant that comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) administered to the individual comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a dimer (*e.g.*, a homodimer). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide), the anti-PD-1 antibody, the anti-HER2 antibody, the 5-fluorouracil, and the platinum-based chemotherapeutic agent are administered simultaneously, concurrently, or sequentially. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide), the anti-PD-1 antibody, the anti-HER2 antibody, the capecitabine, and the platinum-based chemotherapeutic agent are administered simultaneously, concurrently, or sequentially. In some embodiments, the individual receiving treatment has HER2-overexpressing gastric cancer or HER2-overexpressing GEJ cancer. In some embodiments, the gastric cancer or the GEJ cancer is advanced and/or metastatic. In some embodiments, the individual has not received prior treatment for gastric cancer or the GEJ cancer.

[0237] In some embodiments, provided is a method of treating gastric cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1

domain variant and an Fc domain variant administered at a dose between about 10 to about 60 mg/kg once a week (qw). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose between about 10 to about 60 mg/kg once every two weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 10 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 15 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 20 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 30 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 40 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 45 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 50 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 60 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 10 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 15 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 20 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 30 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 40 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 45 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 50 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the

SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 60 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered, *e.g.*, at any of the doses and administration frequencies described herein, in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, as described elsewhere herein) and ramucirumab at a dose of 8 mg/kg once every two weeks, and paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28-day cycle.

[0238] In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks (*e.g.*, an initial dose of 8 mg/kg during week 1 followed by a 6 mg/kg dose given during week two, and a 6 mg/kg dose once every three weeks after the first 6 mg/kg dose). In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks and ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks. In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks, ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks and paclitaxel at a dose of 80 mg/m² on days 1, 8, and 15 every four weeks.

[0239] In some embodiments, provided is a method of treating gastric cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks. In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once

every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks and ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks. In some embodiments, provided is a method of treating cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks, ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks and paclitaxel at a dose of 80 mg/m² on days 1, 8, and 15 every four weeks.

[0240] In some embodiments, provided is a method of treating gastric cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, an initial dose of 6 mg/kg, followed by a 4 mg/kg dose two weeks after the initial 6 mg/kg dose, followed by a 4 mg/kg dose every two weeks after the first 4 mg/kg dose) and ramucirumab at a dose of 8 mg/kg once every two weeks, and paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28-day cycle. In some embodiments, provided is a method of treating gastric adenocarcinoma in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks and ramucirumab at a dose of 8 mg/kg once every two weeks, and paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28-day cycle. In some embodiments, provided is a method of treating gastric esophageal junction adenocarcinoma in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks and ramucirumab at a dose of 8 mg/kg once every two weeks, and paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28-day cycle.

[0241] In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of about 10 to about 60 mg/kg once a week (qw). In some embodiments, the polypeptide comprising the SIRP α D1

domain variant and the Fc domain variant is administered at a dose of about 10 to about 60 mg/kg once every two weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 10 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 15 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 20 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 30 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 40 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 45 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 50 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 60 mg/kg once every week. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 10 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 15 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 20 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 30 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 40 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 45 mg/kg once every two weeks. In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 50 mg/kg once every 2 weeks (q2w). In some embodiments, the polypeptide comprising the SIRP α D1 domain variant and the Fc domain variant is administered at a dose of 60 mg/kg once every 2 weeks (q2w).

[0242] In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks. In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks and ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks. In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 10 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks, ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks and paclitaxel at a dose of 80 mg/m² on days 1, 8, and 15 every four weeks.

[0243] In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks. In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks and ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks. In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 15 mg/kg once every week in combination with trastuzumab at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks, ramucirumab at a dose of 8 mg/kg on days 1 and 15 every four weeks and paclitaxel at a dose of 80 mg/m² on days 1, 8,

and 15 every four weeks.

[0244] In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks and ramucirumab at a dose of 8 mg/kg once every two weeks, and paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28-day cycle. In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, an initial dose of 6 mg/kg, followed by a 4 mg/kg dose two weeks after the initial 6 mg/kg dose, followed by a 4 mg/kg dose every two weeks after the first 4 mg/kg dose) and ramucirumab at a dose of 8 mg/kg once every two weeks, and paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28-day cycle. In some embodiments, provided is a method of treating gastroesophageal junction cancer in an individual, comprising administering to the individual a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant administered at a dose of 30 mg/kg once every two weeks in combination with trastuzumab at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks (*e.g.*, an initial dose of 6 mg/kg, followed by a 4 mg/kg dose two weeks after the initial 6 mg/kg dose, followed by a 4 mg/kg dose every two weeks after the first 4 mg/kg dose) and ramucirumab at a dose of 8 mg/kg once every two weeks, and paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28-day cycle.

[0245] In some embodiments, provided is a method of treating gastric cancer or GEJ cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 50%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an

anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 55%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 60%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 65%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 70%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 75%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 80%. In some embodiments, provided is a

method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 85%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 90%. In some embodiments, provided is a method of treating gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α), (b) an anti-HER 2 antibody, (c) an anti-VEGFR2 antibody, and (d) paclitaxel, wherein, among a population of patients who receive treatment, the overall response rate (ORR) of the population is greater than 95%. In some embodiments, the overall response rate (ORR) is the percent of a population of patients who achieve a partial response (PR) or a complete response (CR) to treatment. In some embodiments, PR and CR are determined according to RECIST criteria (Response Evaluation Criteria in Solid Tumors). Further details regarding RECIST are provided at *e.g.*, [https://ctep\(dot\)cancer\(dot\)gov/protocolDevelopment/docs/recist_guideline.pdf](https://ctep(dot)cancer(dot)gov/protocolDevelopment/docs/recist_guideline.pdf) or [https://recist\(dot\)eortc\(dot\)org](https://recist(dot)eortc(dot)org).

Combination Cancer Therapies Comprising an anti-TROP2 Antibody

[0246] In some embodiments, provided is a method of treating cancer in an individual (*e.g.*, a human individual) that comprises administering to the individual an effective amount of (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) and (b) an anti-TROP2 antibody. In some embodiments, the anti-TROP2 antibody is RS7, which is described in U.S. Patent No. 10,179,171, the contents of which are incorporated herein in their entirety. In some embodiments, the anti-TROP2 antibody is conjugated to a drug (*i.e.*, an antibody-drug conjugate or “ADC”). In some embodiments, the anti-TROP2 ADC is

Sacituzumab govitecan (also known as hRS7-SN38 or IMMU-132), which is described in US 2017/0281791, the contents of which are incorporated herein by reference in their entirety. In some embodiments, the agent is a polypeptide (*e.g.*, fusion polypeptide) comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises a SIRP α D1 domain variant that comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) administered to the individual comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a homodimer. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide), and the anti-TROP2 antibody are administered simultaneously, concurrently, or sequentially. In some embodiments, the cancer is solid tumor, gastric cancer, nasopharyngeal cancer, gallbladder cancer, cervical cancer, extranodal NK/T cell lymphoma, lung cancer, laryngeal squamous cell cancer, colon cancer, Hilar Cholangiocarcinoma, pancreatic cancer, squamous cell carcinoma of the oral cavity, endometrioid endometrial carcinoma, or ovarian carcinoma. In some embodiments, the cancer is characterized by the overexpression of TROP2. In some embodiments, the cancer is not characterized by the overexpression of TROP2.

Methods of Increasing Phagocytosis of a Target Cell

[0247] In some embodiments, provided is a method of increasing phagocytosis of a target cell (*e.g.*, a cancer cell) that comprises contacting the target cell with (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRP α (*e.g.*, hSIRP α) and (b) an anti-TROP2 antibody. In some embodiments, the anti-TROP2 antibody is RS7, which is described in U.S. Patent No. 10,179,171, the contents of which are incorporated herein in their entirety. In some embodiments, the anti-TROP2 antibody is conjugated to a drug (*i.e.*, an antibody-drug conjugate

or “ADC”). In some embodiments, the anti-TROP2 ADC is Sacituzumab govitecan (also known as hRS7-SN38 or IMMU-132), which is described in US 2017/0281791, the contents of which are incorporated herein by reference in their entirety. In some embodiments, the agent is a polypeptide (*e.g.*, fusion polypeptide) comprising a SIRPα D1 domain variant (*e.g.*, a SIRPα D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises a SIRPα D1 domain variant that comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) administered to the individual comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a homodimer. In some embodiments, the target cell is a cancer cell. In some embodiments, the cancer cell is a solid tumor cell, a gastric cancer cell, a nasopharyngeal cancer cell, a gallbladder cancer cell, a cervical cancer cell, an extranodal NK/T cell lymphoma cell, a lung cancer cell, a laryngeal squamous cell cancer cell, a colon cancer cell, a Hilar Cholangiocarcinoma cell, a pancreatic cancer cell, a squamous cell carcinoma cell of the oral cavity, an endometrioid endometrial carcinoma cell, or an ovarian carcinoma cell.

[0248] In some embodiments, provided is a method of increasing phagocytosis of a target cell comprising contacting the target cell with (a) an agent that blocks the interaction between CD47 (*e.g.*, hCD47) and SIRPα (*e.g.*, hSIRPα) and (b) a second agent that is capable of enhancing phagocytosis. In some embodiments, the agent that blocks the interaction between CD47 and SIRPα is a polypeptide (*e.g.*, fusion polypeptide) comprising a SIRPα D1 domain variant (*e.g.*, a SIRPα D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein). In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) comprises a SIRPα D1 domain variant that comprises the amino acid sequence of

SEQ ID NO: 81 or SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) administered to the individual comprises the amino acid sequence of SEQ ID NO: 136 or SEQ ID NO: 135. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) forms a homodimer. In some embodiments, the second agent enhances phagocytosis, *e.g.*, by blocking “don’t eat me” signals. Exemplary agents include, but are not limited to, *e.g.*, an anti-LILRB2 antibody, an anti-LILRB1 antibody, an anti-SIGLEC-10 antibody, an anti-CD24 antibody, an anti-SIRP α antibody, an anti-PD1 antibody (*e.g.*, an anti PD1 antagonist antibody), and an anti-PD-L1 antibody (*e.g.*, an anti PD-L1 antagonist antibody). In some embodiments, the second agent enhances phagocytosis, *e.g.*, by enhancing “eat me” signals. Exemplary agents include, but are not limited to, *e.g.*, BTK activators, TLR agonists, agents that promote the interaction between Mac-1 and SLAMF7, and agents that promote the interaction between calreticulin and LRP1. Additional exemplary agents that enhance phagocytosis include, but are not limited to, *e.g.*, agents that modulate podosome adhesions, agents that modulate the expression level of lamin A, activators of the SHP-1 phosphatase activity, and activators of myosin IIa assembly. In some embodiments, the method comprises contacting the target cell with (a) the polypeptide (*e.g.*, fusion polypeptide) comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein) and (b) and an anti-LILRB2 antibody, an anti-CD24 antibody, or an anti-SIGLEC-10 antibody. In some embodiments, the method comprise contacting the target cell with (a) the fusion polypeptide and (b) a BTK activator. In some embodiments, the method comprises contacting the target cell with (a) the fusion polypeptide and (b) a TLR agonist.

[0249] In some embodiments, the method comprises contacting the target cell with (a) the polypeptide (*e.g.*, fusion polypeptide) comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described

herein) and (b) two or more agents that are capable of enhancing phagocytosis (*e.g.*, including, but not limited to, two or more agents described herein). In some embodiments, the method comprises contacting the target cell with (a) the polypeptide (*e.g.*, fusion polypeptide) comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein), (b) and an anti-LILBR2 antibody, an anti-CD24 antibody, or an anti-SIGLEC-10 antibody, and (c) an anti-PD1 antibody (*e.g.*, an anti-PD-1 antagonist antibody) or an anti-PD-L1 antibody (*e.g.*, an anti-PD-L1 antagonist antibody). In some embodiments, the method comprises contacting the target cell with (a) the fusion polypeptide, (b) an anti-LILBR2 antibody, and (c) an anti-PD1 antibody (*e.g.*, anti-PD-1 antagonist antibody). In some embodiments, the method comprises contacting the target cell with (a) the fusion polypeptide, (b) an anti-LILBR2 antibody, and (c) an anti-PD-L1 antibody (*e.g.* an anti-PD-L1 antagonist antibody).

[0250] In some embodiments, the contacting is performed in vitro. In some embodiments, the contacting is performed in vivo. In some embodiments, the target cell is a cancer cell. In some embodiments, contacting the target cell with (a) the polypeptide comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein) and (b) one or more agents that are capable of enhancing phagocytosis increases phagocytosis of target cells by at least any one of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more than 99% as compared contacting the target cell with one or more agents that are capable of enhancing phagocytosis (*i.e.*, in the absence of the polypeptide comprising a SIRP α D1 domain variant (*e.g.*, a SIRP α D1 domain variant described herein) and an Fc domain variant (*e.g.*, an Fc domain variant described herein)).

Kits and Articles of Manufacture

[0251] In another embodiment of the invention, an article of manufacture or a kit is provided comprising a polypeptide (*e.g.*, a fusion polypeptide described herein) comprising a SIRP α D1 domain variant and an Fc domain variant. In some embodiments, the SIRP α D1 domain variant comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 81 and SEQ ID NO: 85. In some embodiments, the Fc domain variant is (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to

the EU index of Kabat; (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat; (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat. In some embodiments, the Fc domain variant comprises the amino acid sequence of SEQ ID NO: 91. In some embodiments the polypeptide comprises the amino acid sequence of SEQ ID NO: 135 or SEQ ID NO: 136. In some embodiments, the kit or article of manufacture is for use according to a method of treatment provided herein.

[0252] In some embodiments, the kit or article of manufacture further comprises an anti-HER2 antibody (*e.g.*, trastuzumab), an anti-VEGFR2 antibody (*e.g.*, ramucirumab), and paclitaxel. In some embodiments, the kit comprises a package insert or label with instructions for using the polypeptide (*e.g.*, fusion polypeptide) in combination with the anti-HER2 antibody (*e.g.*, trastuzumab), the anti-VEGFR2 antibody (*e.g.*, ramucirumab), and the paclitaxel to treat or delay progression of gastric cancer or gastroesophageal junction (GEJ) cancer in an individual (such as a human individual), *e.g.*, according to a method described herein.

[0253] In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) and the one or more additional anti-cancer agents (*e.g.*, as outlined in the embodiments above) are provided together in the kit. In some embodiments, the polypeptide (*e.g.*, fusion polypeptide) and the one or more additional anti-cancer agents are provided in the same container or separate containers. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation and the label on, or associated with, the container may indicate directions for use. The article of manufacture or kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In some embodiments, the article of manufacture further includes one or more of another agent (*e.g.*, a chemotherapeutic agent, an anti-neoplastic agent, a therapeutic antibody, etc.). Suitable containers for the one or more agents include, for example, bottles, vials, bags and syringes.

[0254] The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0255] The present disclosure will be more fully understood by reference to the following examples. The examples should not, however, be construed as limiting the scope of the present disclosure. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims

Example 1: Anti-tumor activity of DRUG A in combination with trastuzumab and an anti-PD-L1 antibody in a colon cancer model.

[0256] MC38 mouse/human HER2-expressing cells ("MC38 m/h HER2 cells") were generated by infecting MC38 murine colon adenocarcinoma cells with a lentivirus vector encoding a chimera of mouse and human HER2 transmembrane and extracellular domains. MC38 m/h HER2 cells were maintained in DMEM (Thermo Fisher Scientific 11965092) supplemented with 10% FBS, 1% Penicillin-Streptomycin, 1% GlutaMAX and 1mM Sodium Pyruvate (Thermo Fisher Scientific 11360070) at 37°C, 5% CO₂ incubator. All tissue culture was performed under aseptic conditions.

[0257] Prior to implantation, a master cell bank of each cell line was generated to assure that cells used in subsequent experiments were of the same passage number. Cells were harvested and washed two times in 50 mL cold PBS (Life Technologies 10010072). After the final wash, cells were resuspended in PBS or RPMI at 5×10^6 cells/mL for MC38 m/h HER2 cell line. 100 μ L of cell suspension were subcutaneously injected into the right flank of C57BL/6 mice for MC38 m/h HER2. When tumor size reached an average of 65-69 mm³ for MC38 m/h HER2 tumors, the animals were randomized into 8 groups of 10 mice. Each group was assigned to a treatment group outlined in *Table A* below.

Table A. Treatment Groups – MC38 m/h Tumor Model

Treatment Group	Dosing regimen
DRUG A (single agent)	<i>DRUG A (IP)</i> : 30 mg/kg, 2q10d
anti-PD-L1 antibody (single agent)	<i>anti-PD-L1 antibody (IP)</i> : 1 mg/kg, 3q5d
Trastuzumab (single agent)	<i>trastuzumab (IP)</i> : 30 mg/kg, 3q5d
DRUG A + anti-PD-L1 antibody doublet	<i>DRUG A (IP)</i> : 30 mg/kg, 2q10d <i>anti-PD-L1 antibody (IP)</i> : 1 mg/kg, 3q5d
DRUG A + trastuzumab doublet	<i>DRUG A (IP)</i> : 30 mg/kg, 2q10d <i>trastuzumab (IP)</i> : 30 mg/kg, 3q5d
anti-PD-L1 antibody + trastuzumab doublet	<i>anti-PD-L1 antibody (IP)</i> : 1 mg/kg, 3q5d <i>trastuzumab (IP)</i> : 30 mg/kg, 3q5d
DRUG A + anti-PD-L1 antibody + trastuzumab	<i>DRUG A (IP)</i> : 30 mg/kg, 2q10d <i>anti-PD-L1 antibody (IP)</i> : 1 mg/kg, 3q5d
PBS (control)	

[0258] DRUG A is an exemplary fusion protein comprising a SIRP α variant that binds hCD47 with high affinity and an inactive Fc region (*i.e.*, an Fc region that does not exhibit ADCC effector function).

[0259] Tumor volume (mm³) was assessed using a Mitutoyo Digital Caliper (Mitutoyo America, Aurora, Illinois). Tumor volumes and body weights were recorded two or three times per week. Mice exceeding tumor volume of 2000 mm³ or loss of 20% body weight were euthanized according to IACUC guidelines. Tumor volume was calculated as (length x {width x

width}] x 0.5) = volume in mm³). Statistical analysis and p values were calculated using GraphPad Prism Software.

[0260] Chimeric mouse/human HER2, with the extracellular domain from human HER2 and intracellular domain from mouse HER2, was expressed on MC38 colon cells to permit evaluation of trastuzumab's activity against MC38 murine tumors. As shown in FIG 1, Monotherapy with trastuzumab had no effect on tumor growth, while DRUG A monotherapy and anti-PD-L1 antibody monotherapy each had a moderate effect on tumor growth. Treatment with the DRUG A + anti-PD-L1 antibody doublet or the trastuzumab + anti-PD-L1 antibody doublet showed improved tumor growth inhibition as compared to trastuzumab monotherapy, DRUG A monotherapy, and anti-PD-L1 monotherapy. Treatment with the DRUG A + anti-PD-L1 + trastuzumab triple combination showed improved tumor inhibition when compared to each doublet therapy. The effect of the triple combination in inhibiting tumor growth, as compared to the DRUG A + anti-PD-L1 antibody doublet or the trastuzumab + anti-PD-L1 antibody doublet, was most evident on days 19 and 22, which were 3-6 days post final dose. Days 19 and 22 are indicated with "*" in FIG 1. By day 26, the triple combination was minimally better at reducing tumor growth as compared to the DRUG A + anti-PD-L1 antibody doublet or the trastuzumab + anti-PD-L1 antibody doublet. No adverse effects were observed in any of the treatment cohorts in the MC38 m/h HER2 colon tumor models.

Example 2A: Exemplary Clinical Trial to Assess the Anti-Tumor Activity of a DRUG A Combination Therapy in Human Patients

DRUG A, Trastuzumab, Ramucirumab, and Paclitaxel in Gastric or Gastroesophageal Junction (GEJ) Adenocarcinoma

[0261] A clinical trial was performed to assess the safety, tolerability, and efficacy of the combination of DRUG A, trastuzumab, ramucirumab, and paclitaxel in patients with HER2⁺ overexpressing advanced or metastatic gastric or GEJ adenocarcinoma that had progressed during or after prior therapy with trastuzumab and fluoropyrimidine-containing chemotherapy (e.g., fluorouracil); during or after prior therapy with trastuzumab and platinum-containing chemotherapy; or during or after prior therapy with trastuzumab, fluoropyrimidine-containing chemotherapy (e.g., fluorouracil), and platinum-containing chemotherapy. The patients enrolled in the trial were suitable for treatment with trastuzumab. The patients had not received prior

therapy with an anti-CD47 agent or an anti-SIRP α agent.

Example 2B: Preliminary Safety Results from Exemplary Clinical Trials

[0262] One patient with untreated advanced head and neck squamous cell carcinoma (HNSCC) received treatment with DRUG A (10 mg/kg IV QW), pembrolizumab (200 mg IV Q3W), 5-fluorouracil (1,000 mg/m² per day on days 1, 2, 3, 4 Q3W x 6), and carboplatin (AUC = 5 mg/ml/min on Day 1, Q3W x 6). (In expansion studies, cisplatin (100 mg/m² Q3W x 6) or carboplatin (AUC = 5 mg/ml/min on Day 1, Q3W x 6) is administered in combination with DRUG A, pembrolizumab, and fluorouracil. Patients who received carboplatin continued to receive carboplatin for the duration of the expansion studies; patients who received cisplatin continued to receive cisplatin for the duration of the expansion studies).

[0263] Three patients with HER2-positive gastric/gastroesophageal cancer who progressed on prior treatment(s) with trastuzumab, fluorouracil, and a platinum agent received treatment with DRUG A (10 mg/kg IV QW), trastuzumab (8 mg/kg IV for the initial dose, followed by 6 mg/kg Q3W), ramucirumab (8 mg/kg on Days 1 and 15 Q4W), and paclitaxel (80 mg/m² on Days 1, 8, and 15 Q4W).

[0264] Three additional patients with HER2-positive gastric/gastroesophageal cancer who progressed on prior treatment(s) with trastuzumab, fluorouracil, and a platinum agent received treatment with DRUG A (15 mg/kg IV QW), trastuzumab (8 mg/kg IV for the initial dose, followed by 6 mg/kg Q3W), ramucirumab (8 mg/kg on Days 1 and 15 Q4W), and paclitaxel (80 mg/m² on Days 1, 8, and 15 Q4W).

[0265] Initial results suggest that DRUG A, when administered a dose of 10 mg/kg or 15 mg/kg QW in the combination regimens discussed above, is well tolerated with no dose-limiting toxicities to date. Three patients (50%) administered with DRUG A + trastuzumab + ramucirumab + paclitaxel and no patient (0%) administered with DRUG A + pembrolizumab + fluorouracil + carboplatin experienced treatment-related adverse events (TRAEs). There were no dose limiting toxicities in patients receiving DRUG A + pembrolizumab + fluorouracil + carboplatin or DRUG A + trastuzumab + ramucirumab + paclitaxel. There were also no treatment related adverse events (TRAE) that occurred in two or more patients in the following 3 cohorts:

DRUG A (10 mg/kg QW) + pembrolizumab + fluorouracil + carboplatin (N=1)

DRUG A (10 mg/kg QW) + trastuzumab + ramucirumab + paclitaxel (N=3)

DRUG A (15 mg/kg QW) + trastuzumab + ramucirumab + paclitaxel (N=3)

[0266] And finally, there were also no grade 3 or above Treatment related adverse events (TRAE $\geq$ Grade 3) reported in patients treated with DRUG A + pembrolizumab + fluorouracil + carboplatin or DRUG A + trastuzumab + ramucirumab + paclitaxel.

Example 2C: Preliminary Efficacy Results from the Exemplary Clinical Trials Described in Example 2A

[0267] The patient with untreated advanced head and neck squamous cell carcinoma (HNSCC) who received treatment with DRUG A, pembrolizumab, 5-fluorouracil, and a platinum agent at the dosages and administration schedule described in *Example 2B* achieved partial response (PR) based on investigator-assessed response using RECIST v1.1 criteria.

[0268] Among the three patients with HER2-positive gastric/gastroesophageal cancer who received treatment with DRUG A (10 mg/kg QW), trastuzumab, ramucirumab, and paclitaxel, (see *Example 2B*) two were not yet evaluable. One patient achieved PR based on investigator-assessed response using RECIST v1.1 criteria.

[0269] Among the three patients with HER2-positive gastric/gastroesophageal cancer who received treatment with DRUG A (15 mg/kg QW), trastuzumab, ramucirumab, and paclitaxel, (see *Example 2B*) two were not yet evaluable. One patient achieved PR based on investigator-assessed response using RECIST v1.1 criteria. Low rates of cytopenias were observed.

[0270] DRUG A in combination with pembrolizumab, 5-fluorouracil, and a platinum agent showed clinical activity in the treatment of advanced 1L HNSCC (*i.e.*, as a first treatment in patients with advanced HNSCC who have not received prior therapy for HNSCC.) DRUG A in combination with trastuzumab, ramucirumab, and paclitaxel showed clinical activity in the treatment of advanced ≥ 2 L gastric/gastroesophageal cancer (*i.e.*, as a treatment in patients who have received at least one prior therapy for gastric or GEJ cancer).

[0271] Results from pharmacodynamics analyses indicated that near complete CD47 target occupancy (also known as receptor occupancy) was maintained throughout the DRUG A dosing interval when combined with chemotherapy-containing regimens.

Example 2D: Additional Results from the Exemplary Clinical Trials Described in Example 2A

[0272] CD47 is a myeloid checkpoint up-regulated by tumors to evade the anticancer immune response. DRUG A is an exemplary high affinity CD47-blocking fusion protein with an inactive Fc region designed to safely enhance anticancer therapeutics (Kauder *et al.* (2018) *PLoS ONE*. 13(8): e0201832; Chow *et al.* (2020) *Journal of Clinical Oncology*. 38:15_suppl, 3056-3056; and Lakhani *et al.* (2021) *Lancet Oncology*. 22(12):1740-1751). DRUG A in combination with standard chemotherapy and antibody regimens was evaluated in patients with advanced HER2-positive gastric cancer (GC) or with head and neck squamous cell carcinoma (HNSCC).

Methods

[0273] Patients with previously treated advanced HER2-positive GC received DRUG A (A) 10 mg/kg QW or 15 mg/kg QW in combination with trastuzumab (T) + ramucirumab (ram) + paclitaxel (pac) as 2nd or later-line treatment. The GC patients had progressed during or following a prior fluoropyrimidine therapy (or a fluoropyrimidine-containing therapy). GC patients who had progressed during or following a prior therapy with trastuzumab and/or a platinum-based chemotherapeutic agent were included. Patients with untreated advanced HNSCC received DRUG A (A) 10 mg/kg QW or 15 mg/kg QW in combination with pembrolizumab (P) + 5FU + platinum (cisplatin or carboplatin) as 1st line therapy. The primary endpoint was dose limiting toxicity (DLT). Tumor response, pharmacokinetic (PK), and pharmacodynamic (PD) markers were assessed in all patients.

Results

[0274] Fifty-five patients were enrolled in the study. Patients' baseline characteristics are shown in *Table B*:

Table B: Baseline Characteristics

	<i>DRUG A + trastuzumab + chemo ≥2L GC (N=14)</i>	<i>DRUG A + pembrolizumab + chemo 1L HNSCC (N=5)</i>
<i>Median age, years (range)</i>	63 (36-83)	61 (45-63)

<i>Sex, n</i>	M	10	4
	F	4	1
<i>Race, n</i>	Asian	11	4
	White	3	1
	Other	-	-
<i>ECOG PS, n</i>	0	5	4
	1	9	1
<i>Progressed upon prior anti-HER2 Therapy, n (%)</i>		13 (93)	N/A
<i>Progressed upon ≥ 2 prior anti-HER2 therapy n (%)</i>		1 (7.1)	N/A
<i>Progressed upon prior CPI Therapy, n (%)</i>		1 (7.1)	0 (0)
<i>Visceral distant metastasis, n (%)</i>		13 (93)	1 (20)

[0275] 1 patients with ≥ 2 L GC received A + T + ram + pac and were evaluated for safety. No dose-limiting toxicities (DLTs) were reported, and the DRUG A maximum administered dose was 15 mg/kg QW. Of the 9 patients who experienced any adverse event, 8 patients reported treatment-related adverse events (TRAE). The most common TRAEs were low grade diarrhea, fatigue, pruritus/urticaria and rash (each n =21%). TRAEs $\geq$ Grade 3 severity were of low frequency. There were no treatment related SAEs reported amongst GC patients treated with A + T + ram + pac. Among the 11 GC patients who received DRUG A at 15 mg/kg qw + trastuzumab + ramucirumab + paclitaxel, 7 demonstrated partial response, 3 demonstrated stable disease, and 1 demonstrated progressive disease. Among the 3 patients who received DRUG A at 10 mg/kg qw + trastuzumab + ramucirumab + paclitaxel, 2 demonstrated partial response, and one demonstrated stable disease.

[0276] Three patients with previously untreated HNSCC were administered A + P + 5FU + platinum, as described above. No DLTs were reported. Three pts experienced any adverse

events (AE), none were treatment-related. The HNSCC patient who received DRUG A at 15 mg/kg qw + pembrolizumab + 5-fluorouracil + a platinum-based chemotherapeutic agent was CPI naïve and demonstrated partial response. Of the three patients who received DRUG A at 10 mg/kg qw + pembrolizumab + 5-fluorouracil + a platinum-based chemotherapeutic agent, all were CPI naïve. One patient demonstrated complete response, one patient demonstrated partial response, and one demonstrated progressive disease.

[0277] The clinical activity of DRUG A chemotherapy combinations in response evaluable patients are summarized in *Table C* below:

Table C: Responses to DRUG A Chemotherapy Combinations

Patient / Treatment	N	ORR (95% CI)	Median Follow-up* (95% CI)
≥ 2L Gastric Cancer / DRUG A + trastuzumab + ramucirumab + paclitaxel	14	64.3% [38.8% ; 83.7%]	5.3[2.8 ; 6.7]
DRUG A (15mg/kg qw)	11	63.6% [35.4% ; 84.8%]	4.2[2.4 ; 6.2]
DRUG A (10mg/kg qw)	3	66.7% [20.8% ; 93.9%]	8.9[5.1 ; 9.6]
1L HNSCC / DRUG A + pembrolizumab + 5FU + platinum	4	75% [30.0% ; 95.0%]	5.0 [1.3 ; 8.8]
DRUG A (15mg/kg qw)	1	100% [20.5 ; 100%]	1.6[1.3 ; 1.9]
DRUG A (10mg/kg qw)	3	66.7% [20.8% ; 93.9%]	5.3[5.0 ; 8.8]

[0278] Initial DRUG A combination PK and CD47 target occupancy are similar to that of single agent administration. Near complete (80%-100%) CD47 target occupancy is maintained throughout DRUG A dosing interval when combined with chemotherapy-containing regimens. Circulating immune cell profiles (CD4⁺ T cells, CD8⁺ T cells, CD19⁺ B cells, and CD16⁺CD56⁺

NK cells) are generally unchanged following DRUG A combined with chemotherapy-containing regimens. DRUG A PK following combination therapies with pembrolizumab or trastuzumab is comparable, with and without chemotherapy.

Conclusions

[0279] Preliminary data indicated that DRUG A is well tolerated and can be safely combined with the anticancer antibody + multi-agent chemotherapy regimens studied with no maximum tolerated dose reached. The maximum administered dose of DRUG A in combination was 15 mg/kg QW.

[0280] DRUG A demonstrates initial ORR of 64% in patients with ≥ 2 L HER2 positive GC in combination with trastuzumab and ramucirumab + paclitaxel that compares favorably with the clinical experience of ramucirumab + paclitaxel in patients whose disease has progressed upon prior trastuzumab-containing regimens.

[0281] DRUG A demonstrates initial anti-cancer activity including complete and partial objective responses in combination with pembrolizumab + 5FU + platinum in patients who have not received prior treatment for their advanced HNSCC.

[0282] Preliminary pharmacokinetics and pharmacodynamic analysis demonstrates no impact of the combination partners upon DRUG A exposure levels with full CD47 receptor occupancy.

Example 2E: A Phase 1 study of DRUG A, an agent that blocks the interaction between CD47 and SIRP α , in combination with trastuzumab, ramucirumab, and paclitaxel in patients with 2nd line HER2-positive advanced gastric or gastroesophageal (GC) cancer

[0283] Data provided herein further supports use of DRUG A as a single agent and in combination with established anti-cancer agents, namely, the anti-HER2 antibody trastuzumab, the anti-VEGFR2 antibody ramucirumab, and paclitaxel. DRUG A was administered at a dose of 10 mg/kg (mpk) QW or 15 mg/kg (mpk) QW in combination with trastuzumab, ramucirumab, and paclitaxel to patients with HER2-overexpressing advanced or metastatic gastric cancer (GC) or gastroesophageal junction (GEJ) adenocarcinoma that has progressed on or after treatment with a prior HER2-directed agent and fluoropyrimidine-containing therapy or with a prior platinum-containing chemotherapy. The patients had received one or two prior treatments for

GC or GEJ adenocarcinoma. The maximum administered dose of DRUG A in combination was 15 mg/kg QW. There were no dose limiting toxicities on study deaths, or DRUG A-related serious adverse events (SAEs) in patients receiving the DRUG A + trastuzumab + ramucirumab + paclitaxel combination treatment.

[0284] Briefly, FIGs. 2A and 2B provide the results of phase 1 clinical study of combination therapy of DRUG A in combination with (a) an anti-HER2 antibody (trastuzumab), (b) an anti VEGFR antibody (ramucirumab), and (c) paclitaxel. FIG. 2A, provides a graph of the best % change in baseline measurable lesions in each study participant. 3/18 patients achieved stable disease (SD), 12/18 achieved partial response (PR), and one patient achieved complete response (CR). FIG. 2B provides a graph showing the % change in baseline measurable lesions in each study participant as a function of time.

[0285] *Table D* below shows further analyses of the clinical activity of DRUG A combinations in response evaluable patients with ≥ 2 HER2 positive GC or GEJ adenocarcinoma. The table shows the overall response rate (ORR) among patients receiving treatment with DRUG A + trastuzumab + ramucirumab + paclitaxel was about 72%.

Table D: Overall Response Rate (ORR), Duration of Response (DOR), Progression Free Survival (PFS) Rates and Overall Survival (OS) Rates among Gastric/Gastroesophageal Cancer Patients treated with DRUG A + Trastuzumab + Ramucirumab + Paclitaxel

Population	N (eval)	ORR (%) [95% CI]	DOR (months) [95% CI]	PFS (months) [95% CI]	PFS rate at 6 months [95% CI]	OS (months) [95% CI]	OS rate at 12 months [95% CI]	Follow up (months) [95% CI]
≥ 2L Gastric /GEJ trastuzumab + ramucirumab + paclitaxel + 10 mg/kg DRUG A or 15 mg/kg DRUG A	18	72.2 [49.1%, 87.5%]	NR	9.1 [3.8; NR]	74.5%	NR	75.8%	10.5 [4.8; 12.5]
≥ 2L Gastric /GEJ trastuzumab + ramucirumab + paclitaxel + 10 mg/kg DRUG A	3	66.7 [20.8%, 93.9%]	NR	NR	100%	NR	66.7%	14.3 [12.0; NR]
≥ 2L Gastric /GEJ trastuzumab + ramucirumab + paclitaxel + 15mg/kg DRUG A	15	73.3 [48.1%, 89.1%]	NR	NR	68.3%	NR	80.8%	9.4 [4.2; 12.5]
≥ 2L Gastric /GEJ trastuzumab + 10 mg/kg DRUG A	19*	21.1 [8.5%, 43.3%]	8.7 [5.6; NR]	2.2 [1.9; 5.5]	16.7%	8.1 [3.4; 12.6]	38.2%	27.0 [NR]

NR = Not reached

*One patient discontinued prior to first imaging assessment with symptoms consisted with progression and was not evaluable for response, per protocol

[0286] Preliminary data suggest that DRUG A can be safely combined with trastuzumab, ramucirumab and paclitaxel with no maximum tolerated dose reached.

[0287] Preliminary PK/PD analysis demonstrate no impact of the combination partners upon DRUG A exposure levels with full CD47 receptor occupancy.

[0288] DRUG A in combination with trastuzumab, ramucirumab and paclitaxel demonstrates an initial ORR of 72.2% and estimated overall survival (OS) at 12 months of 75.8% in patients with ≥ 2 L HER2 positive GC or GEJ adenocarcinoma that has progressed after a prior trastuzumab-containing regimen. This compares favorably with both RAINBOW (See Wilke et al., Lancet October 2014) and DESTINY-01 (See Enhertu package insert, and Shitara et al., NEJM June 18, 2020) clinical trial historical controls.

[0289] Updated data from patients receiving DRUG A + trastuzumab after their tumors have progressed upon prior trastuzumab therapy suggests clinical activity beyond that expected from either trastuzumab or chemotherapy alone.

Example 3: A Phase 2/3 Study of DRUG A in Patients With Advanced HER2-Overexpressing Gastric/Gastroesophageal Junction Adenocarcinoma

[0290] The data described herein supports further trials to test efficacy of the combination therapies described herein. This is a randomized phase 2 (open-label) / 3 (double-blind), international, multi-center study of patients with metastatic HER2-overexpressing gastric/GEJ adenocarcinoma that has progressed on or after prior HER2-directed therapy and fluoropyrimidine- or platinum-containing chemotherapy and are suitable for chemotherapy (2nd-line or 3rd-line). Approximately 450 adult patients are expected to be enrolled in the study across both phases.

[0291] Conditions: Gastric Cancer; Gastroesophageal Junction Adenocarcinoma; Gastric Adenocarcinoma

[0292] Experimental: Phase 2 – Arm A: DRUG A - 30 mg/kg Q2W IV, trastuzumab (initial dose of 6 mg/kg followed by 4 mg/kg) Q2W IV, ramucirumab 8 mg/kg Q2W IV, and paclitaxel 80 mg/m² IV Days 1, 8, and 15 of a 28-day cycle. Active Comparator: Phase 2 - Arm B Trastuzumab (initial dose of 6 mg/kg followed by 4 mg/kg) Q2W IV, ramucirumab 8 mg/kg Q2W IV, and paclitaxel 80 mg/m² IV Days 1, 8, and 15 of a 28-day cycle.

[0293] Experimental: Phase 3 - Arm A: DRUG A 30 mg/kg Q2W IV, trastuzumab (initial dose of 6 mg/kg followed by 4 mg/kg) Q2W IV, ramucirumab 8 mg/kg Q2W IV, and paclitaxel 80 mg/m² IV Days 1, 8, and 15 of a 28-day cycle. Active Comparator: Phase 3 - Arm B Ramucirumab 8 mg/kg Q2W IV and paclitaxel 80 mg/ m² IV Days 1, 8, and 15 of a 28-day cycle.

[0294] Outcome Measures: Primary Outcome Measures include for Phase 2 an objective response rate per RECIST 1.1 and for Phase 3 an overall survival.

[0295] The following are eligibility criteria for the study: Eligibility Minimum Age: 18 Years; Maximum Age: none. Sex: All; Accepts Healthy Volunteers: No.

[0296] Inclusion Criteria: HER2-overexpressing advanced or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma that has progressed on or after a prior HER2-directed agent and fluoropyrimidine- or platinum-containing chemotherapy (2nd-line or 3rd-line); Adequate Bone Marrow Function; Adequate Renal & Liver Function.; Adequate Performance Status.

[0297] Exclusion Criteria: Patients with known symptomatic CNS metastases or leptomeningeal disease requiring steroids, Prior treatment with any anti-CD47 or anti-SIRP α agent, Prior treatment with ramucirumab.

Example 4: Effects of DRUG B in Combination with Anti-Mouse VEGFR-2, Paclitaxel and Trastuzumab in a Syngeneic Murine Model of HER2⁺ Colorectal Carcinoma

Overview

[0298] DRUG B, a murine surrogate for DRUG A, comprises a SIRP α variant that exhibits high affinity for murine CD47 ("mCD47") and an inactive Fc domain (*i.e.*, an Fc domain that does not exhibit effector function). DRUG B, was evaluated in a quadruplet combination treatment with with trastuzumab (*i.e.*, an anti-human HER2 antibody), anti-mouse VEGFR-2 (*i.e.*, a murine ramucirumab surrogate), and paclitaxel in a chimeric mouse/human HER2 CT26 (CT26 m:h HER2), murine colorectal carcinoma model expressing mouse and human HER2. The triplet treatment of trastuzumab, paclitaxel, and anti-mouse VEGFR-2, exhibited marginal tumor growth inhibition. However, the addition of DRUG B to the triplet regimen significantly enhanced tumor growth inhibition and prolonged survival.

[0299] The therapeutic effect of DRUG B in the absence of trastuzumab was also evaluated. The combination of DRUG B with paclitaxel and anti-mouse VEGFR-2 doublet therapy in CT26 murine colorectal carcinoma model did not show enhanced anti-tumor activity as compared to paclitaxel and anti-mouse VEGFR-2 therapy.

[0300] These data demonstrate that inhibiting the CD47-SIRP α axis with DRUG B potentiates the anti-tumor efficacy of trastuzumab, anti-mouse VEGFR-2 and paclitaxel treatments in a HER2 expressing tumor model. In the absence of trastuzumab, DRUG B does not enhance anti-tumor activity of paclitaxel and anti-mouse VEGFR-2 doublet therapy.

[0301] DRUG A was shown to bridge innate and adaptive immunity to reduce immune suppression and activate the adaptive immune response in an antigen-specific manner. DRUG A treatment resulted in an increased anti-tumor response, significant tumor growth inhibition and prolonged survival in combination with anti-PD-1 and anti-PD-L1 antibodies in both checkpoint inhibitor sensitive syngeneic murine models of cancer (*i.e.*, using the murine CT26 colon carcinoma cell line and the murine MC38 colon adenocarcinoma cell line) and a checkpoint inhibitor resistant syngeneic murine model of cancer (*i.e.*, using the murine 4T1 mammary cancer cell line). See Kauder *et al.* (2018) PLoS One. 2018;13(8):e0201832.

[0302] In HER2 positive gastric/gastroesophageal junction (G/GEJ) cancer, the necessary pro-phagocytic signal is provided by using trastuzumab to specifically target the HER2 antigen while engaging Fc γ R on macrophages with its active Fc IgG1 domain. This selective pro-phagocytic signal in combination DRUG A's blockade of the anti-phagocytic myeloid checkpoint signal maximizes the macrophage's G/GEJ cancer specific ADCP activity. By providing blockade of the anti-phagocytic CD47-SIRP α interaction in conjunction with the tumor targeting and macrophage Fc γ R engagement provided by trastuzumab, DRUG A in combination, is able to deliver anti-tumor activity even in clinical settings where trastuzumab has been shown to have minimal activity, such as in the treatment of HER2-positive gastric cancer with trastuzumab and paclitaxel in the second line setting in disease that has progressed upon trastuzumab (T-ACT trial; Makiyama *et al.* (2020) *Clin Oncol.* 8(17): 1919-1927).

Study Objectives

[0303] The experiments in this Example were performed to characterize the *in vivo* anti-tumor activity for DRUG B, a murine surrogate for DRUG A, in combination with paclitaxel and

anti-mouse VEGFR-2, in the presence or absence of trastuzumab in a syngeneic murine colorectal carcinoma model.

Materials and Methods

[0304] Reagents used in this Example include those listed in Table E below:

Table E. Reagents

Reagent	Source	Lot	Isotype
DRUG B	ALX Oncology	ES20201005	Mouse IgG1 N297A AAA
Anti-mouse VEGFR-2 clone DC101 (Ramucirumab surrogate)	BioXell	796321M1	Rat IgG1, k
Anti-HER2 (Trastuzumab)	Genentech	3094840	Human IgG1
Paclitaxel	Med Chem Express	CS-1145	NA

Animals for In Vivo Studies

[0305] BALB/c were purchased from Charles River Laboratories International (Hollister, CA). All animals were housed in pathogen free facility in accordance with IACUC guidelines. Animals used for all studies were 6-8 weeks old.

Cell Lines

[0306] CT26 m:h HER2 was generated by the transduction of CT26 (ATCC CRL-2638) with lentivirus expressing murine HER2 engrafted with the extracellular domain of trastuzumab epitope.

[0307] CT26 and CT26 m:h HER2 were cultured in complete RPMI-1640 medium consisting of RPMI- 1640 (Thermo Fisher Scientific 11875119) medium supplemented with 10% fetal bovine serum (FBS) (Millipore TMS-013B), 1% Penicillin-Streptomycin (Thermo Fisher Scientific 15140163) and 1% GlutaMAX (Thermo Fisher Scientific 35050061) at 37°C, 5% CO₂ incubator.

Subcutaneous Tumor Models in Mice

[0308] All tissue culture was performed under aseptic conditions. CT26 and CT26 m:h HER2 cells were harvested and washed two times in 50 mL cold PBS (Thermo Fisher Scientific

10010072). After the final wash, cells were resuspended in serum free RPMI-1640 at 20×10^6 cells/mL of CT26 or CT26 m:h HER2. 100 mL of cell suspension were subcutaneously injected into the right flank of BALB/c mice.

[0309] In the CT26 tumor model, when tumors reached an average of $47\text{--}85 \text{ mm}^3$, the animals were randomized into groups of eight mice and dosing was initiated. All treatment agents were administered intraperitoneally (IP). Paclitaxel was administered one day prior to rest of the regimen at 20 mg/kg for a total of three doses every five days. DRUG B was administered five times five days apart at 30 mg/kg. Anti-mouse VEGFR-2 was administered five times every 2-3 days at 40 mg/kg.

[0310] In the CT26 m:h HER2 tumor model, when tumors reached an average of $45\text{--}73 \text{ mm}^3$, the animals were randomized into groups of 10 mice and dosing was initiated. All treatment agents were administered IP. Paclitaxel was administered one day prior to rest of the regimen at 20 mg/kg for a total of three doses every three days. DRUG B was administered four times five days apart at 30 mg/kg. Anti-mouse VEGFR-2 was administered five times four days apart at 40 mg/kg. Trastuzumab was administered four times five days apart at 30 mg/kg.

[0311] Tumor volumes (mm^3) were assessed using a Mitutoyo Digital Caliper (Mitutoyo America, Aurora, Illinois). Tumor volumes and body weights were recorded 1-3 times per week. Tumor volume is calculated as $([\text{length} \times \{\text{width} \times \text{width}\}] \times 0.5) = \text{volume in } \text{mm}^3$. P-values were calculated using student's t-test for tumor volume and survival was calculated using Log-rank (Mantel-Cox) test using Prism 9 software (GraphPad). Mice exceeding tumor volume of 2000 mm^3 and loss of 20% body weight were euthanized in accordance with the IACUC guidelines.

Results and Discussion

DRUG B Does Not Enhance Anti-Tumor Activity in Combination with Anti-Mouse VEGFR-2 and Paclitaxel compared to Anti-Mouse VEGFR-2 + Paclitaxel Doublet in a CT26 Syngeneic Tumor Model

[0312] In a CT26 colon carcinoma tumor model, animals treated with the doublet regimen of 20 mg/kg paclitaxel and 40 mg/kg anti-mouse VEGFR-2 exhibited sub-optimal tumor growth inhibition. As shown in **FIG 3**, a triplet combination comprising 20 mg/kg paclitaxel, 40 mg/kg

anti-mouse VEGFR-2, and 30 mg/kg DRUG B did not inhibit tumor growth compared to the doublet regimen of paclitaxel, and anti-mouse VEGFR-2 alone.

Addition of DRUG B to Trastuzumab, Anti-Mouse VEGFR-2, and Paclitaxel Triplet Enhances Anti-Tumor Response Compared to Triplet in a CT26 m:h HER2-expressing colon carcinoma tumor model

[0313] In a CT26 m:h HER2-expressing colon carcinoma tumor model, animals treated with the triplet regimen of 30 mg/kg trastuzumab, 20 mg/kg paclitaxel, and 40 mg/kg anti-mouse VEGFR-2 exhibited marginal tumor growth inhibition. The addition of 30 mg/kg DRUG B to the trastuzumab + paclitaxel + anti-mouse VEGFR-2 triplet significantly enhanced tumor growth inhibition as compared to the triplet in the absence of DRUG B (day 26 unpaired t-test, $p < 0.0022$). On day 26, the quadruplet cohort had three out of ten animals with complete tumor eradication. No tumor eradication was observed in the triplet or PBS cohorts. See FIG 4A, which shows tumor growth $\pm$ SEM of $n = 10$ mice per cohort. Additionally, DRUG B in combination with the trastuzumab, anti-mouse VEGFR-2, and paclitaxel triplet regimen significantly increased survival compared to the triplet or PBS cohorts alone. (Log-rank (Mantel-Cox) test, $p < 0.0001$). See FIG 4B.

[0314] These results demonstrate that in a CT26 m:h HER2-expressing colon carcinoma tumor model, DRUG B in combination with trastuzumab, paclitaxel, and anti-mouse VEGFR-2 significantly increased anti-tumor response, enhanced tumor growth inhibition resulting in complete tumor eradication, and prolonged survival in a subset of treated animals.

Conclusions

[0315] The anti-tumor effect of DRUG B in combination with paclitaxel and anti-mouse VEGFR2 (*i.e.*, without trastuzumab) was evaluated in a CT26 syngeneic mouse tumor model. The DRUG B + paclitaxel, + anti-mouse VEGFR-2 triplet therapy did not show enhanced anti-tumor activity as compared to paclitaxel and anti-mouse VEGFR-2 doublet therapy.

[0316] The anti-tumor effect of DRUG B in combination with trastuzumab, paclitaxel, and anti-mouse VEGFR2 was evaluated in a CT26 m:h HER2-expressing colon carcinoma tumor model. The quadruple combination significantly increased tumor growth inhibition as compared to the trastuzumab, paclitaxel, and anti-mouse VEGFR2 triplet, resulting in complete tumor eradication and prolonged survival in a subset of treated animals. The trastuzumab, paclitaxel,

and anti-mouse VEGFR-2 triplet did not show significant tumor growth inhibition as compared to PBS control.

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- [0325] The preceding Examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

CLAIMS

1. A method of treating cancer in an individual, comprising administering to the individual an effective amount of: (a) a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant, (b) an anti-HER2 antibody, (c) an anti-VEGF2 antibody, and (d) paclitaxel;
wherein the SIRP α D1 domain variant comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85;
wherein the Fc domain variant is
 - (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat;
 - (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat;
 - (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or
 - (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat,wherein the cancer is gastric cancer (GC) or gastroesophageal junction (GEJ) cancer, and wherein the individual has received at least one prior therapy for the gastric or the GEJ cancer.
2. The method of claim 1, wherein the individual has received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, or with an anti HER2 antibody and a platinum-based chemotherapy agent.
3. The method of claim 1 or 2, wherein the anti-HER2 antibody is trastuzumab.
4. The method of any one of claims 1-3, wherein the anti-VEGF antibody is ramucirumab.
5. The method of any one of claims 1-4, wherein the gastric cancer or the GEJ cancer is HER2⁺ gastric cancer or HER2⁺ GEJ cancer.
6. The method of any one of claims 1-5 wherein the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered at a dose of 10 mg/kg once a week.

7. The method of any one of claims 1-5 wherein the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered at a dose of 15 mg/kg once a week.
8. The method of any one of claims 1-5, wherein the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered at a dose of 30 mg/kg once a week.
9. The method of any one of claims 1-5, wherein the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered at a dose of 10 mg/kg once every two weeks.
10. The method of any one of claims 1-5, wherein the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered at a dose of 15 mg/kg once every two weeks.
11. The method of any one of claims 1-5, wherein the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant is administered at a dose of 30 mg/kg once every two weeks.
12. The method of any one of claims 1-11, wherein the trastuzumab is administered at an initial dose of 8 mg/kg followed by 6 mg/kg once every three weeks.
13. The method of any one of claims 1-11, wherein the trastuzumab is administered at an initial dose of 6 mg/kg followed by 4 mg/kg once every two weeks.
14. The method of any one of claims 1-13, wherein the paclitaxel is administered at a dose of 80 mg/m² once a week for three weeks of every four week cycle.
15. The method of any one of claims 1-14, wherein the paclitaxel is administered at a dose of 80 mg/m² on Days 1, 8, and 15 of every 28-day cycle.
16. The method of any one of claims 1-15, wherein, among a population of patients receiving treatment, the overall response rate (ORR) of the population is greater than 65%.

17. A kit comprising a polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant in a pharmaceutically acceptable carrier, for use in combination with an anti-HER2 antibody, an anti-VEGFR2 antibody, and paclitaxel;
- wherein the SIRP α D1 domain variant comprises the amino acid sequence of SEQ ID NO: 81 or SEQ ID NO: 85;
- wherein the Fc domain variant is
- (i) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, wherein numbering is according to the EU index of Kabat;
- (ii) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, wherein numbering is according to the EU index of Kabat;
- (iii) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, and delG236 mutations, wherein numbering is according to the EU index of Kabat; or
- (iv) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, wherein numbering is according to the EU index of Kabat,
- and wherein the kit comprises instructions for administering the polypeptide comprising a SIRP α D1 domain variant and an Fc domain variant in combination with the anti-HER2 antibody, the anti-VEGFR2 antibody, and the paclitaxel to an individual with gastric cancer or gastroesophageal (GEJ) cancer who has received at least one prior treatment for gastric cancer or GEJ cancer.
18. The kit of claim 17, wherein the gastric cancer or GEJ cancer is HER2⁺ gastric cancer or HER2⁺ GEJ cancer.
19. The kit of claim 17 or 18, wherein the anti-HER2 antibody is trastuzumab.
20. The kit of any one of claims 17-19, wherein the anti-VEGFR2 antibody is ramucirumab.
21. The kit of any one of claims 17-20, wherein the individual received prior therapy with an anti-HER2 antibody, with an anti-HER2 antibody and a fluoropyrimidine, or with an anti-HER2 antibody and a platinum-based chemotherapy agent.