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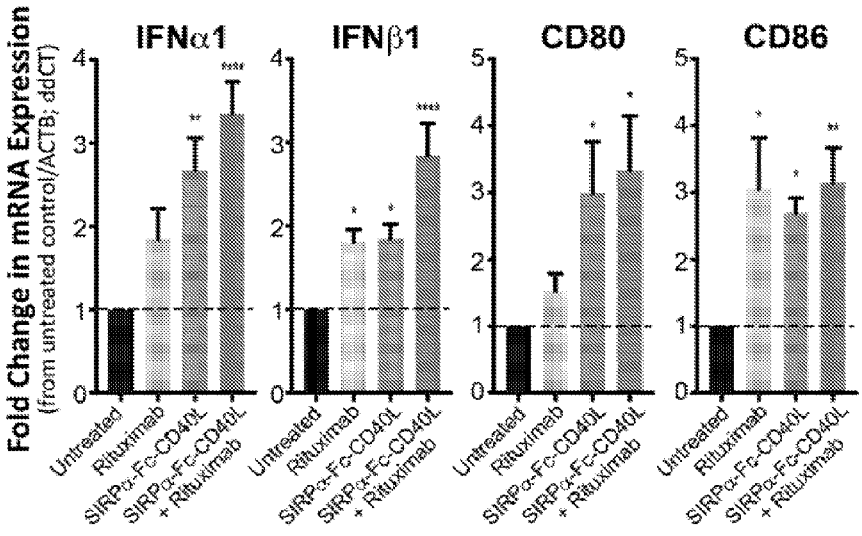
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(54) Title: COMBINATION THERAPIES COMPRISING SIRP ALPHA-BASED CHIMERIC PROTEINS

FIG. 8D



(57) Abstract: The present invention relates to, *inter alia*, combinations of compositions which include chimeric proteins that find use in methods for treating disease, such as immunotherapies for cancer and autoimmunity.

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COMBINATION THERAPIES COMPRISING SIRP ALPHA-BASED CHIMERIC PROTEINS

PRIORITY

This application claims the benefit of, and priority to, U.S. Provisional Application No. 62/724,600, filed Aug 29, 2018; U.S. Provisional Application No. 62/734,951, filed Sept 21, 2018; U.S. Provisional Application No. 62/793,235, filed January 16, 2019; U.S. Provisional Application No. 62/832,830, filed April 11, 2019; U.S. Provisional Application No. 62/890,217, filed August 22, 2019; the contents of each of which is herein incorporated by reference in its entirety.

TECHNICAL FIELD

The present invention relates to, *inter alia*, combinations of compositions which include chimeric proteins that find use in methods for treating disease, such as immunotherapies for cancer and autoimmunity.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

This application contains a sequence listing. It has been submitted electronically *via* EFS-Web as an ASCII text file entitled "SHK-013PC_SequenceListing_ST25". The sequence listing is 57,420 bytes in size, and was created on August 29, 2019. The sequence listing is hereby incorporated by reference in its entirety.

BACKGROUND

The immune system is central to the body's response to cancer cells and disease-causing foreign entities. Many cancers, however, have developed mechanisms to avoid the immune system by, for instance, delivering or propagating immune inhibitory signals. Additionally, many anti-cancer therapeutics do not directly stimulate and/or activate the immune response. Current combination immunotherapy with bispecific antibodies, linked scFv's, or T cell engagers have not been able to both block checkpoints (immune inhibitory signals) and agonize (stimulate) TNF receptors. This is likely because these molecules lose target avidity when engineered to bind multiple targets with monovalent antigen binding arms. Thus, there remains a need to develop therapeutics that, at least, are endowed with multiple functionalities but still retain target avidity – for instance, reverse immune inhibitory signals and stimulating an anti-cancer immune response.

SUMMARY

Accordingly, in various aspects, the present invention provides compositions and methods that are useful for cancer immunotherapy. For instance, the present invention, in part, relates to methods for treating cancer comprising administering (either simultaneously or sequentially) at least one antibody directed to an immune checkpoint molecule; a stimulator of interferon genes (STING) agonist; and/or one or more chimeric proteins, in which each chimeric protein is capable of blocking immune inhibitory signals and/or stimulating immune activating signals.

An aspect of the present invention is a method for treating a cancer in a subject in need thereof. The method comprises steps of providing the subject a first pharmaceutical composition and providing the subject a second pharmaceutical composition. The first pharmaceutical composition comprises a heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. The second pharmaceutical composition comprises an antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), or human epidermal growth factor receptor 2 (Her2), or and capable of, respectively, inhibiting the interaction of CD20, EGFR, or Her2, with one or more of its ligands.

Another aspect of the present invention is method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. In this aspect, the subject has undergone or is undergoing treatment with an antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), or human epidermal growth factor receptor 2 (Her2), or and capable of, respectively, inhibiting the interaction of CD20, EGFR, or Her2, with one or more of its ligands.

Yet another aspect of the present invention is a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), or human epidermal growth factor receptor 2 (Her2), or and capable of, respectively, inhibiting the interaction of CD20, EGFR, or Her2, with one or more of its ligands. In this aspect, the subject has undergone or is undergoing treatment with: a heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In an aspect, the present invention provides a method for treating a cancer in a subject in need thereof. The method comprising steps of providing the subject a first pharmaceutical composition comprising an antibody that is capable of

binding cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and providing the subject a second pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In another aspect, the present invention provides a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. In this aspect, the subject has undergone or is undergoing treatment with an antibody that is capable of binding cytotoxic T lymphocyte-associated antigen 4 (CTLA-4).

In yet another aspect, the present invention provides a method for treating a cancer in a subject comprising: providing the subject a pharmaceutical composition comprising an antibody that is capable of binding cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). In this aspect, the subject has undergone or is undergoing treatment with a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

An aspect of the present invention is a method for treating a cancer in a subject in need thereof. The method comprising steps of: providing the subject a first pharmaceutical composition comprising a stimulator of interferon genes (STING) agonist, and providing the subject a second pharmaceutical composition comprising a heterologous chimeric protein. In this aspect, the heterologous chimeric protein comprises: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and (c) a linker linking the first domain and the second domain.

Another aspect of the present invention is a method for treating a cancer in a subject. The method comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and (c) a linker linking the first domain and the second domain. In this aspect, the subject has undergone or is undergoing treatment with a stimulator of interferon genes (STING) agonist.

Yet another aspect of the present invention is a method for treating a cancer in a subject. The method comprising providing the subject a pharmaceutical composition comprising a stimulator of interferon genes (STING) agonist. In this aspect, the subject has undergone or is undergoing treatment with a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and (c) a linker linking the first domain and the second domain.

In an aspect, the present invention provides a method for treating a cancer in a subject in need thereof. The method comprising: providing the subject a first pharmaceutical composition comprising a heterologous chimeric protein and providing the subject a second pharmaceutical composition comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand and/or capable of inhibiting the interaction of PD-1 with one or more of its ligands. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, , and (c) a linker linking the first domain and the second domain.

In another aspect, the present invention provides a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. In this aspect, the subject has undergone or is undergoing treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand and/or capable of inhibiting the interaction of PD-1 with one or more of its ligands.

In yet another aspect, the present invention provides a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand and/or capable of inhibiting the interaction of PD-1 with one or more of its ligands. In this aspect, the subject has undergone or is undergoing treatment with a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the chimeric proteins of the present invention and/or chimeric proteins used in methods of the present invention eliminate or reduce side effects associated with disrupting the SIRP1α/CD47 signaling axis. In embodiments, the present chimeric proteins or methods utilizing the same eliminate or reduce hematological adverse effects. In embodiments, the present chimeric proteins or methods utilizing the same eliminate or reduce the extent of reductions in the number of circulating red blood cells and platelets, hemolysis, hemagglutination, thrombocytopenia, and/or anemia. In embodiments, the present chimeric proteins or methods utilizing the same demonstrate comparatively less hematological adverse effects than an anti-CD47 antibody.

Any aspect or embodiment disclosed herein can be combined with any other aspect or embodiment as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A to **FIG. 1D** show schematic illustrations of Type I transmembrane proteins (**FIG. 1A** and **FIG. 1B**, left proteins) and Type II transmembrane proteins (**FIG. 1A** and **FIG. 1B**, right proteins). A Type I transmembrane protein and a Type II transmembrane protein may be engineered such that their transmembrane and intracellular domains are omitted and the transmembrane proteins' extracellular domains are adjoined using a linker sequence to generate a single chimeric protein. As shown in **FIG. 1C** and **FIG. 1D**, the extracellular domain of a Type I transmembrane protein, *e.g.,* SIRPα(CD172a), and the extracellular domain of a Type II transmembrane protein, *e.g.,* CD40L, and OX40L, are combined into a single chimeric protein. **FIG. 1C** depicts the linkage of the Type I transmembrane protein and the Type II transmembrane protein by omission of the transmembrane and intracellular domains of each protein, and where the liberated extracellular domains from each protein have been adjoined by a linker sequence. The extracellular domains in this depiction may include the entire amino acid sequence of the Type I protein (*e.g.,* SIRPα(CD172a)) and/or Type II protein (*e.g.,* CD40L, OX40L, LIGHT) which is typically localized outside the cell membrane, or any portion thereof which retains binding to the intended receptor or ligand. Moreover, the chimeric protein used in a method of the present invention comprises sufficient overall flexibility and/or physical distance between domains such that a first extracellular

domain (shown at the left end of the chimeric protein in **FIG. 1C** and **FIG. 1D)** is sterically capable of binding its receptor/ligand and/or a second extracellular domain (shown at the right end of the chimeric protein in **FIG. 1C** and **FIG. 1D)** is sterically capable of binding its receptor/ligand. **FIG. 1D** depicts adjoined extracellular domains in a linear chimeric protein wherein each extracellular domain of the chimeric protein is facing "outward".

FIG. 2 shows immune inhibitory and immune stimulatory signaling that is relevant to the present invention (from Mahoney, *Nature Reviews Drug Discovery* 2015:14;561-585).

FIG. 3A (top) is an image showing the predicted tertiary structure of human SIRPα-Fc-CD40L (RaptorX; University of Chicago) and **FIG. 3A** (bottom) shows a Western blot analysis of SIRPα-Fc-CD40L performed by probing purified protein with human anti-SIRPα, anti-Fc, and anti-CD40L, under non-reducing and reducing conditions, and ± the deglycosylase PNGase F. **FIG. 3B** shows an electron microscopy image indicating hexameric structure of the SIRPα-Fc-CD40L. Scale is shown and yellow arrows correspond with each identified monomer. A schematic of the hexamer species is shown to the right, depicting dimerization of the Fc domain and trimerization of the CD40L domain. **FIG. 3C** shows a functional dual ELISA using capture with recombinant hCD40 followed by detection with recombinant hCD47-His and then anti-His-HRP. **FIG. 3D** shows single-sided ELISA detection of SIRPα-Fc-CD40L using recombinant Fc, CD47, and CD40 capture. **FIG. 3E** shows the use of Surface Plasmon Resonance (SPR) to determine on-, off-, and binding affinities for SIRPα-Fc-CD40L to recombinant hCD47, hCD40, hFcγR1A, and FcRn. Recombinant hSIRPα-Fc, hCD40L-Fc, and hIgG1 were used as positive controls. **FIG. 3F** shows verification of human CD47 and human CD40 expression in CHO-K1 cells used to assess binding to SIRPα-Fc-CD40L. In both panels, the CHOK1 Parental is on the left and the CHOK1/hCD47 is on the right. **FIG. 3G** shows Flow cytometry-based binding of SIRPα-Fc-CD40L to CHOK1 cells engineered to stably express human CD47 or human CD40. For both graphs, the CHOK1 Parental is on the bottom. **FIG. 3H** shows a competition ELISA in which the disruption of binding of recombinant hSIRPα-Fc to plate-bound hCD47 was assessed in the presence or absence of SIRPα-Fc-CD40L or a human CD47 blocking antibody. In this figure, the control is the top curve, the SIRPα-Fc-CD40L is the middle curve, and the anti-CD47 is the bottom curve.

FIG. 4A shows western blot analysis of the murine SIRPα-Fc-CD40L surrogate with antibodies detecting mSIRPα, mFc, and mCD40L under non-reducing, reducing, and PNGase F/reducing conditions. **FIG. 4B** shows dual functional ELISA of the murine SIRPα-Fc-CD40L surrogate, demonstrating simultaneous binding to recombinant mouse CD47 and CD40.

FIG. 5A shows data from CHO-K1 cells that were stably engineered to express human CD40 and an NFkB-luciferase reporter from Promega. Cells were cultured with a dose titration of recombinant human CD40L-his or SIRPα-Fc-CD40L, and luminescence was read on a luminometer after 6 hours. The left histograms are (-) Control, middle histograms are hCD40L-His and the right histograms are SIRPα-Fc-CD40L. **FIG. 5B** shows data from non-canonical NIK/NFkB reporter U2OS cells (expressing human CD40) were obtained from DiscoverX and cultured with a titration of

recombinant human CD40L-Fc, an agonist hCD40 antibody, or SIRPα-Fc-CD40L; and luminescence was determined after 6 hours. CD8-depleted PBMC from 33-50 distinct human donors were cultured with media only, the neoantigen positive control KLH, the clinical stage non-activating control Exenatide, or .3, 3, 30, or 300 nM of SIRPα-Fc-CD40L. At 10 μg/ml on the X-axis, the samples are, top to bottom: hCD40L-Fc, SIRPα-Fc-CD40L, ahCD40 (HB14) ad (-) Control. **FIG. 5C** shows a murine version of the NFκB-luciferase reporter assay in CHO-K1 cells developed to express murine CD40 and an NFκB-luciferase reporter. The left histograms are (-) control, second from left are FC-mCD40L, second from right are anti-mCD40 (FGK4.5) and right are mSIRPα-Fc-CD40L.

FIG. 6A shows data from CD8+ T cell-depleted PBMC cultured in the presence of a dose-titration of hSIRPα-Fc-CD40L. Here, proliferation was assessed *via* [^{3}H]-Thymidine incorporation on days 5, 6, and 7. The order of samples (in triplicate) along the X-axis is media only, KLH, Exenatide, .3 nm SIRPα-Fc-CD40L, 3 nm SIRPα-Fc-CD40L, 30 nm SIRPα-Fc-CD40L and 300 nm SIRPα-Fc-CD40L. **FIG. 6B**: shows data on day 8, for IL-2 positive cells. Here, proliferation was assessed by ELISpot. The order of samples (in triplicate) along the X-axis is media only, KLH, Exenatide, .3 nm SIRPα-Fc-CD40L, 3 nm SIRPα-Fc-CD40L, 30 nm SIRPα-Fc-CD40L and 300 nm SIRPα-Fc-CD40L.

FIG. 7A and **FIG. 7B** show confocal microscopy images of fluorescent markers for CD11b (**FIG. 7A**) and FITC stain (tumor cells, **FIG. 7B**). **FIG. 7C**, **FIG. 7D,** and **FIG.** 7E each show confocal microscopy images of fluorescent markers for tumor cells (FITC stain). **FIG. 7F**, **FIG. 7G,** and **FIG. 7H** each show a confocal microscopy images of fluorescent markers for tumor cells (FITC stain, **FIG. 7F**), macrophages (DAPI stain, **FIG. 7G**), and macrophages (DAPI stain, stitched image, **FIG. 7H**).

FIG. 8A shows *in vitro* engulfment of tumor cells by macrophages when treated with control IgGs, an anti-CD20 antibody (Rituximab), the SIRPα(CD172a)-Fc-CD40L chimeric protein, or a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody. **FIG. 8B** is a chart that quantifies the phagocytosis index of the experiments shown in **FIG. 8A**. **FIG. 8C** shows increased expression of INFα1 and IFNβ1, synthesis of IFNβ, and phosphorylated IRF3 in cells treated with the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody. **FIG. 8D**: Macrophage:Toledo lymphoma co-cultures were harvested 2 hours into phagocytosis assays in the presence of rituximab (.06 mM), SIRPα-Fc-CD40L (1 mM), or the combination of both agents. Macrophages were isolated by CD11b+ FACS, RNA was prepared, cDNA synthesized, and gene expression (IFNa1, IFNb1, CD80, and CD86) assessed by qRT-PCR. Fold change was calculated using DDCT and values obtained from the housekeeping gene ACTB, and the corresponding values from untreated samples. **FIG. 8E** shows phagocytosis quantitation of Raji cells by human macrophages using flow cytometry. **FIG. 8F** shows a phagocytosis index of human monocyte derived macrophages and Toledo lymphoma cells that were co-cultured with an IgG negative control, monotherapy SIRPα-Fc-CD40L and rituximab, and the combination of these agents. After 2 hours, co-cultures were isolated from tissue culture vessels and were stained with a fluorescently conjugated CD11b antibody to discriminate macrophages. Cells were then assessed by flow cytometry for the co-localization of fluorescent signal from both cells

types. Identical assays were set up where macrophages were pre-incubated for 1 hour with 20 µg/mL of a commercially available Fc block cocktail or with 20 µg/mL of a CD40 blocking antibody or with 5 µg/mL of a calreticulin blocking peptide. **FIG. 8G:** RAW 264.7-Lucia ISG cells were cultured with A20 lymphoma cells in the presence of 50 mg/mL of mSIRPα-Fc-CD40L, recombinant Fc-mCD40L, mSIRPα-Fc, or their combination, 1 mg/mL anti-mCD20, or the combination of mSIRPα-Fc-CD40L and anti-mCD20. After 24-hours type I interferon induced luminescence was assessed using a luminometer. The maximum luminescence (RLU) from each experiment was set to 1, and all other samples were normalized accordingly. Values from repeat experiments are shown. **FIG. 8H** shows a murine version of the phagocytosis assay using bone marrow derived macrophages co-cultured with A20 lymphoma or WEHI3 leukemia cells, in the presence of mSIRPα-Fc-CD40L or anti-CD47.

FIG. 9A shows images and graphs of SIRPα-Fc-CD40L stimulated macrophage phagocytosis. For each panel, the samples are (left to right): untreated, sheep RBCs, αCD47 (MIAP301), αSIRPα (P84), mSIRPα-Fc-CD40L, mSIRPα-Fc-CD40L (24Hrs), and αCD47 (MIAP301) (24 hrs). **FIG. 9B** is a quantitation of *in vivo* dendritic cell activation corresponding to **FIG. 9A**. Shown is the absolute percent of CD4+ and CD8+ dendritic cells; also gated on CD11c and DC1R2. In **FIG. 9B**, for each panel, the samples are (left to right): untreated, sheep RBCs, αCD47 (MIAP301), αSIRPα (P84), SIRPα-Fc-CD40L (150 µg), SIRPα-Fc-CD40L (300 µg), SIRPα-Fc-CD40L (300 µg) (24Hrs), and αCD47 (MIAP301) (24 hrs).

FIG. 10A is a schematic illustration showing the design of an *in vitro* phagocytosis assay with human donor macrophages and human tumor cell lines (*e.g.,* Raji cells). **FIG. 10B** shows an *in vitro* phagocytosis assay with human donor macrophages and Raji cells treated with a variety of protein and antibody combinations that included control IgGs with an anti-CD20 antibody (Rituximab), an anti-CD47 antibody (CC9, Celgene), the SIRPα(CD172a)-Fc-CD40L chimeric protein, and/or pembrolizumab (KEYTRUDA/MK 3475, Merck). **FIG. 10C** shows an *in vitro* phagocytosis assay with human donor macrophages and Raji cells were treated with a variety of chimeric protein and antibody combinations that included control IgGs with an anti-CD20 antibody (Rituximab), the SIRPα(CD172a)-Fc-CD40L chimeric protein, and/or pembrolizumab (KEYTRUDA/MK 3475, Merck).

FIG. 11 shows an *in vitro* phagocytosis assay with human donor macrophages and Raji cells where Fc receptors on macrophages were either blocked ("with Fc block") or not blocked ("without Fc block"). The Raji cells were treated with a variety of chimeric protein and antibody combinations that included control IgGs with an anti-CD20 antibody (Rituximab), an anti-CD47 antibody (CC9, Celgene), the SIRPα(CD172a)-Fc-CD40L chimeric protein, and/or pembrolizumab (KEYTRUDA/MK 3475, Merck). The order of samples (pairs of histograms) from left to right mirrors the order in the legend top to bottom (*e.g.,* No Drug is far left and SIRPα-Fc-CD40L + Pembro is far right).

FIG. 12A and **FIG. 12B** show an IFNα (**FIG. 12A**) and IFNβ (**FIG. 12B**) ELISA on 24 hour phagocytosis co-cultures. In these figures, the term "ARC" refers to the SIRPα(CD172a)-Fc-CD40L chimeric protein.

FIG. 13A to **FIG. 13C** show *in vitro* engulfment of tumor cells by macrophages when untreated or treated with control IgGs, an anti-EGFR antibody (cetuximab), the SIRPα(CD172a)-Fc-CD40L chimeric protein, or a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-EGFR antibody; the tumor cells used were, respectively, a high EGFR-expressing skin cancer cell line (A431), a high EGFR-expressing lung cancer cell line (HCC827), and a low EGFR-expressing Chronic myeloid leukemia (CML) cell line (K562).

FIG. 14A and **FIG. 14B** show *in vitro* engulfment of tumor cells by macrophages when untreated or treated with control IgGs, an anti-Her2 antibody (trastuzumab), the SIRPα(CD172a)-Fc-CD40L chimeric protein, or a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-Her2 antibody; the tumor cells used were, respectively, a high Her2-expressing breast cancer cell line (HCC1954) and a low Her2-expressing breast cancer cell line (MCF7).

FIG. 15A is table showing anti-tumor treatment schedules for the *in vivo* experiments disclosed **FIG. 15B** to **FIG. 15D** and **FIG. 16A** and **FIG. 16B**. **FIG. 15B** shows *in vivo* reductions in tumor volume size for control treatments and **FIG. 15C** and **FIG. 15D** show *in vivo* reductions in tumor volume size resulting from methods of cancer treatments according to the present invention. In **FIG. 15B**, at time point 18 days, the order of curves top to bottom is Vehicle, anti-PD1, anti-OX40, anti-CTLA4, and SIRPα-Fc-CD40L. In **FIG. 15C**, at time point 18 days, the order of curves top to bottom is Vehicle, anti-CTLA4 then anti-PD1, anti-CTLA4 then anti-OX40, anti-CTLA4 then SIRPα-Fc-CD40L. In **FIG. 15D**, the top curve is Vehicle and bottom curve is SIRPα-Fc-CD40L.

FIG. 16A shows *in vivo* reductions in tumor volume size for control treatments and **FIG. 16B** show *in vivo* reductions in tumor volume size resulting from methods of cancer treatments according to the present invention. In **FIG. 16A**, at day 20, the curves are, top to bottom, vehicle (IP), vehicle (IT), anti-PD1, SIRPα-Fc-CD40L, DMXAA and anti-OX40. In **FIG. 16B**, at day 20, the curves are, top to bottom, vehicle (IP), vehicle (IT), DMXAA then anti-PD1, DMXAA then SIRPα-Fc-CD40L, and DMXAA then anti-OX40.

FIG. 17A shows *in vivo* reductions in tumor volume size resulting from the methods of cancer treatments according to the present invention. In the left panel, at day 15, the curves are, from top to bottom: vehicle, the anti-CTLA-4 antibody, and the SIRPα-Fc-CD40L chimeric protein ("ARC"); in the right panel, at day 25, the curves are, from top to bottom: the SIRPα-Fc-CD40L chimeric protein ("ARC") then the anti-CTLA-4 antibody, the SIRPα-Fc-CD40L chimeric protein ("ARC") with the anti-CTLA-4 antibody, and the anti-CTLA-4 antibody then the SIRPα-Fc-CD40L chimeric protein ("ARC"). **FIG. 17B** shows Kaplan-Meier plots of the percent survival days after tumor inoculation for the different combinations shown in **FIG. 17A**. **FIG. 17C** includes data relevant to the graphs of **FIG. 17A** and **FIG. 17B**. In these figures, the term "ARC" refers to the SIRPα(CD172a)-Fc-CD40L chimeric protein.

FIG. 18A shows *in vivo* reductions in tumor volume size resulting from the methods of cancer treatments according to the present invention. In the left panel, at day 15, the curves are, from top to bottom: vehicle, the anti-PD-1 antibody, and the SIRPα-Fc-CD40L chimeric protein ("ARC"); in the right panel, at day 25, the curves are, from top to bottom:

the SIRPα-Fc-CD40L chimeric protein ("ARC") then the anti-PD-1 antibody, the anti-PD-1 antibody then the SIRPα-Fc-CD40L chimeric protein ("ARC"), and the SIRPα-Fc-CD40L chimeric protein ("ARC") with the anti-PD-1 antibody. **FIG. 18B** shows Kaplan-Meier plots of the percent survival days after tumor inoculation for the different antibody combinations shown in **FIG. 18A**. **FIG. 18C** includes data relevant to the graphs of **FIG. 18A** and **FIG. 18B**. In these figures, the term "ARC" refers to the SIRPα(CD172a)-Fc-CD40L chimeric protein.

FIG. 19 shows graphs of treating mice with anti-PD1 or anti-CTLA-4 stimulates expansion of CD40-expressing immune cells. CT26 bearing mice treated with two IP doses of 100 µg of either anti-CTLA-4 or anti-PD1 given on days 7 and 9, were euthanized 11 days after tumor inoculation. Tumors were isolated, homogenized, and assessed by flow cytometry for populations of CD40+ dendritic cells (CD11c+), B cells (CD19+), and T cells (CD3+); and the absolute MFI levels of MHC I, MHC II, and CD47. In these histograms, each set of three bars is from left to right: vehicle, anti-PD-1 antibody, and anti-CTLA-4 antibody.

FIG. 20A: mice were inoculated subcutaneously on the rear flank with $5x10^5$ CT26 cells on day 0, and then treated with 2 doses of the indicated antibody or mSIRPα-Fc-CD40L (all by IP injection on days 5 & 7 once the tumors established and were ~30 mm^3, a schematic of the treatment schedule is shown. STV stands for 'starting tumor volume', on the day that treatment began. At day 20, the order of curves is from(top to bottom: vehicle, anti-CD40, anti-CD47, anti-CD40/anti-CD47 combo, and mSIRPα-Fc-CD40L.**FIG. 20B**: a cohort of mice was euthanized 13 days following tumor inoculation, and spleens/tumors were excised, dissociated, and subjected to antigen-specific CD8+ T cell assessment using tetramer reagents to the dominant antigen in CT26 cells (AH1 tetramer). **FIG. 20C**: a similar CT26 experiment was initiated as described in **FIG. 20A** above, however mice were pre-treated on days -1, 1, and 10 with IP injections of 100 µg of either or both CD4 / CD8 depleting antibodies. Mice were inoculated with CT26 tumors on day 0 and treated at a later starting date than **FIG. 20A** with mSIRPα-Fc-CD40L ARC on days 7, 9, and 11. In **FIG. 20C**, at day 15, the order of curves is from top to bottom: vehicle, mSIRPα-Fc-CD40L + αCD4/αCD8, mSIRPα-Fc-CD40L + αCD8, mSIRPα-Fc-CD40L + αCD4, and mSIRPα-Fc-CD40L. In **FIG. 20D** and **FIG. 20E,** BALB/C mice were inoculated with WEHI3 tumors (**FIG. 20D**) or A20 tumors (**FIG. 20E**) subcutaneously on the rear flank, and then treated *via* IP injection with anti-CD20 or mSIRPα-Fc-CD40L on days 7, 9, and 11 (WEHI3) or days 10, 12, and 14 (A20); when tumors were established and reached approximately 57-60 mm^3. A cohort of mice were pre-treated with an interferon alpha receptor 1 (IFNAR1) blocking antibody (500 mg by IP injection) on days -1, 1, and 10 (WEHI3) or on days -1, 1, and 13 (A20). **FIG. 20F** to **FIG. 20H** are graphs showing blockade of CD4, CD8, and IFNAR1. Peripheral blood analysis by flow cytometry of CD4 (**FIG. 20F**), CD8 (**FIG. 20G**), and IFNAR1 (**FIG. 20H**) depletion following depleting antibody treatment corresponding to **FIG. 20C** to **FIG. 20E**. Samples were normalized to untreated animals.

FIG. 21 is a graph showing cynomolgus macaques that were treated with vehicle, or .1, 1, 10, and 40 mg/kg of SIRPα-Fc-CD40L. Blood chemistry analysis assessed peripheral erythrocyte counts, hemoglobin levels, and hematocrit. In addition, the fold-change in lymphocyte count from pre-dose to 24 hours post-dose is shown.

FIG. 22A shows an *in vitro* hemolysis assay using human donor red blood cells (RBCs) treated with a titration of the positive control Triton X-100, a CD47 blocking antibody previously shown to induce RBC lysis (clone CC2C6), and a titration of 3 separate lots of SIRPα-Fc-CD40L. **FIG. 22B** is a graph showing RBCs and test agents that were incubated at 37°C for 24 hours and then the media was assessed at OD490 for changes in absorbance due to hemoglobin being released from lysing RBCs. **FIG. 22C** is a graph showing a decrease in overall CD45+ peripheral lymphocytes was observed 24 hours following a single IP injection of mSIRPα-Fc-CD40L (300 µg). **FIG. 22D** are graphs showing that peripheral blood was isolated from mice receiving three IP doses (300 µg) of the murine SIRPα-Fc-CD40L surrogate (arrows). Cell populations were assessed by flow cytometry and included CD20+ B cells, CD11C+, CD4+/CD11c+, and CD8+/CD11c+ dendritic cells. No significant differences were observed in mice treated with an interferon alpha-receptor 1 depleting antibody (anti-IFNAR1).

FIG. 23A to **FIG. 23C** are schematic illustrations showing proposed SIRPα-Fc-CD40L mechanism of action. In **FIG. 23A**, tumor expressed CD47 can provide a 'do not eat me' signal to antigen presenting cells (APCs) through the binding of SIRPα. **FIG. 23B** shows that SIRPα-Fc-CD40L can relieve this inhibitory signal while simultaneously providing an 'eat me' signal *via* costimulation of CD40 by CD40L, together enhancing tumor phagocytosis, APC activation, increased antigen processing/presentation, and induction of an anti-tumor antigen-specific CD8+ T cell response. **FIG. 23C** shows that consistent with others developing agents to target the CD47/SIRPα axis, combining SIRPα-Fc-CD40L with targeted ADCP competent antibodies potentiates their phagocytosis activity.

FIG. 24 shows western blot analysis of the murine SIRPα-Fc-OX40L surrogate with antibodies detecting mSIRPα, mFc, and mOX40L under non-reducing, reducing, and PNGase F/reducing conditions.

FIG. 25A shows *in vivo* reductions in tumor volume size resulting from the methods of cancer treatments according to the present invention. In the top panel, at day 7, the order of curves is from top to bottom: vehicle, SIRPα-Fc, SIRPα-Fc + OX40L-Fc, OX40L-Fc, SIRPα-Fc-OX40L + anti-CTLA4, SIRPα-Fc-OX40L, and SIRPα-Fc-OX40L + anti-PD1; in the bottom panel, at day 5, the order of curves is from top to bottom: vehicle, anti-CTLA4, and anti-PD1. **FIG. 25B** shows Kaplan-Meier plots of the percent survival days after tumor inoculation for the different combinations shown in **FIG. 25A**. **FIG. 25C** includes data relevant to the graphs of **FIG. 25A** and **FIG. 25B**.

FIG. 26A shows *in vivo* reductions in tumor volume size resulting from the methods of cancer treatments according to the present invention. In the top panel, at day 10, the order of curves is from top to bottom: vehicle, anti-PD-1 antibody, SIRPα-Fc-LIGHT, and SIRPα-Fc-LIGHT + anti-PD1 antibody. **FIG. 26B** shows Kaplan-Meier plots of the percent survival days after tumor inoculation for the different combinations shown in **FIG. 26A**. **FIG. 26C** and **FIG. 26D** include data relevant to the graphs of **FIG. 26A** and **FIG. 26B**.

DETAILED DESCRIPTION

The present invention is based, in part, on the discovery of methods for treating cancer comprising administering (either simultaneously or sequentially) at least one antibody directed to an immune checkpoint molecule; a stimulator of interferon genes (STING) agonist; and/or one or more chimeric proteins, in which each chimeric protein is capable of blocking immune inhibitory signals and/or stimulating immune activating signals.

Importantly, since the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention disrupt, block, reduces, inhibit, and/or sequester the transmission of immune inhibitory signals, *e.g.,* originating from a cancer cell that is attempting to avoid its detection and/or destruction and/or enhance, increase, and/or stimulate the transmission of an immune stimulatory signal to an anti-cancer immune cell, the methods can provide an anti-tumor effect by multiple distinct pathways. By treating cancer *via* multiple distinct pathways, the methods of the present invention are more likely to provide any anti-tumor effect in a patient and/or to provide an enhanced anti-tumor effect in a patient. Moreover, since the methods operate by multiple distinct pathways, they can be efficacious, at least, in patients who do not respond, respond poorly, or become resistant to treatments that target one of the pathways. Thus, a patient who is a poor responder to treatments acting via one of the two pathways, can receive a therapeutic benefit by targeting multiple pathways.

Without wishing to be bound by theory, the SIRPα(CD172a)-Fc-CD40L chimeric proteins of the present invention and/or the SIRPα(CD172a)-Fc-CD40L chimeric proteins used in methods of the present invention may operate according to the following mechanisms. First, the SIRPα(CD172a)-Fc-CD40L chimeric proteins may directly activate antigen presenting cells by binding to CD40 on APCs. Here, an advantage may be antigen-specific CD8 stimulation and/or programming of immune memory. When used in a combination, antibodies related to checkpoint molecules may increase CD40 target density for SIRPα(CD172a)-Fc-CD40L costimuation and upregulation of antigen presentation machinery. Second, the SIRPα(CD172a)-Fc-CD40L chimeric proteins may directly block CD47 inhibition by tumor cells blocking and sequestering CD47 on tumor cells. Here, an advantage may be enhanced tumor phagocytosis and increased antigen cross-presentation. . When used in a combination, antibody-dependent cellular cytotoxicity-related antibodies increase targeted tumor phagocytosis, antigen cross-presentation and anti-tumor response. **FIG. 23A** to **FIG. 23C** are schematic illustrations showing proposed SIRPα-Fc-CD40L mechanisms of action.

In embodiments, the chimeric proteins of the present invention and/or chimeric proteins used in methods of the present invention eliminate or reduce side effects associated with disrupting the SIRP1α/CD47 signaling axis. In embodiments, the present chimeric proteins or methods utilizing the same eliminate or reduce hematological adverse effects. In embodiments, the present chimeric proteins or methods utilizing the same eliminate or reduce the extent of reductions in the number of circulating red blood cells and platelets, hemolysis, hemagglutination, thrombocytopenia, and/or

anemia. In embodiments, the present chimeric proteins or methods utilizing the same demonstrate comparatively less hematological adverse effects than an anti-CD47 antibody.

Antibodies

The methods of the present invention comprise methods for treating cancer, which, in embodiments, comprise administering an immunotherapy comprising an antibody capable of binding an immune checkpoint molecule.

The antibody may be selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In embodiments, the antibody is a monoclonal antibody, *e.g.,* a humanized monoclonal antibody.

In embodiments, the antibody is capable of binding PD-1 or a PD-1 ligand, *e.g.,* selected from the group consisting of nivolumab (ONO 4538, BMS 936558, MDX1106, OPDIVO (Bristol Myers Squibb)), pembrolizumab (KEYTRUDA/MK 3475, Merck), and cemiplimab ((REGN-2810). Such an antibody is, optionally, capable of inhibiting the interaction of PD-1 with one or more of its ligands.

In embodiments, the antibody is capable of binding CTLA-4, *e.g.,* selected from the group consisting of YERVOY (ipilimumab), 9D9, tremelimumab (formerly ticilimumab, CP-675,206; MedImmune), AGEN1884, and RG2077.

In embodiments, the antibody is capable of binding OX40, *e.g.,* selected from the group consisting of GBR 830 (GLENMARK), MEDI6469 (MEDIMMUNE), OX86, BMS-986178, PF-04518600, INCAGN01949, MEDI0562, GSK3174998, and PF-04518600.

STING Agonists

The methods of the present invention comprise methods for treating cancer, which, in embodiments, comprise administering a pharmaceutical composition comprising a stimulator of interferon genes (STING) agonist.

In embodiments, the STING Agonist is selected from the group consisting of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), MIW815(ADU-S100), CRD5500, MK-1454, SB11285, IMSA101, and any STING agonist described in US20140341976, US20180028553, US20180230178, US9549944, WO2015185565, WO2016120305, WO2017044622, WO2017027645, WO2017027646, WO2017093933, WO2017106740, WO2017123657, WO2017123669, WO2017161349, WO2017175147, WO2017175156, WO2017176812, WO2018009466, WO2018045204, WO2018060323, WO2018098203, WO2018100558, WO2018138684, WO2018138685, WO2018152450, WO2018152453, WO2018172206, WO2018198084, WO2018234805, WO2018234807, WO2018234808, WO2019023459, WO2019046496, WO2019046498, WO2019046500, WO2019074887, WO2019079261, WO2019118839, WO2019125974, or WO2019160884, he contents of which are incorporated herein by reference in their entireties.

Chimeric Proteins

The methods of the present invention comprise methods for treating cancer, which, in embodiments, comprise administering a pharmaceutical composition comprising a chimeric protein capable of blocking immune inhibitory signals and/or stimulating immune activating signals.

Chimeric proteins used in methods of the present invention comprise a general structure of: N terminus – (a) – (b) – (c) – C terminus, where (a) is a first domain comprising an extracellular domain of Type I transmembrane protein, (b) is a linker adjoining the first domain and the second domain, *e.g.,* the linker comprising at least one cysteine residue capable of forming a disulfide bond and/or comprising a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of a Type II transmembrane protein; wherein the linker connects the first domain and the second domain. Alternately, a chimeric proteins used in methods of the present invention comprise a general structure of: N terminus – (a) – (b) – (c) – C terminus, where (a) is a first domain comprising an extracellular domain of Type I transmembrane protein, (b) is a linker adjoining the first domain and the second domain, *e.g.,* the linker comprising at least one cysteine residue capable of forming a disulfide bond and/or comprising a hinge-CH2-CH3 Fc domain, and (c) is a second domain comprising an extracellular domain of another Type I transmembrane protein; wherein the linker connects the first domain and the second domain.

Transmembrane proteins typically consist of an extracellular domain, one or a series of transmembrane domains, and an intracellular domain. Without wishing to be bound by theory, the extracellular domain of a transmembrane protein is responsible for interacting with a soluble receptor or ligand or membrane-bound receptor or ligand (*i.e.,* a membrane of an adjacent cell) in the extracellular environment. Without wishing to be bound by theory, the trans-membrane domain(s) is responsible for localizing the transmembrane protein to the plasma membrane. Without wishing to be bound by theory, the intracellular domain of a transmembrane protein is responsible for coordinating interactions with cellular signaling molecules to coordinate intracellular responses with the extracellular environment (or visa-versa).

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

There are generally two types of single-pass transmembrane proteins: Type I transmembrane proteins which have an extracellular amino terminus and an intracellular carboxy terminus (see, **FIG. 1A**, left protein) and Type II transmembrane proteins which have an extracellular carboxy terminus and an intracellular amino terminus (see, **FIG. 1A**, right protein). Type I and Type II transmembrane proteins can be either receptors or ligands. For Type I

transmembrane proteins (*e.g.,* SIRPα(CD172a)), the amino terminus of the protein faces outside the cell, and therefore contains the functional domains that are RESPONSIBLE for interacting with other binding partners (either ligands or receptors) in the extracellular environment (see, **FIG. 1B**, left protein). For Type II transmembrane proteins (*e.g.,* CD40L OX40L, and LIGHT), the carboxy terminus of the protein faces outside the cell, and therefore contains the functional domains that are responsible for interacting with other binding partners (either ligands or receptors) in the extracellular environment (see, **FIG. 1B**, right protein). Thus, these two types of transmembrane proteins have opposite orientations to each other relative to the cell membrane.

Chimeric proteins used in methods of the present invention comprise an extracellular domain of a Type I transmembrane protein, *e.g.,* SIRPα(CD172a), and an extracellular domain of a Type II transmembrane protein selected from CD40L, OX40L, and LIGHT. Thus, a chimeric protein used in a method of the present invention comprises, at least, a first domain comprising the extracellular domain of SIRPα(CD172a), which is connected – directly or *via* a linker – to a second domain comprising the extracellular domain of CD40L, OX40L, or LIGHT. As illustrated in **FIG. 1C** and **FIG. 1D**, when the domains are linked in an amino-terminal to carboxy-terminal orientation, the first domain is located on the "left'" side of the chimeric protein and is "outward facing" and the second domain is located on "right" side of the chimeric protein and is "outward facing".

Other configurations of first and second domains are envisioned, *e.g.,* the first domain is inward facing and the second domain is outward facing, the first domain is outward facing and the second domain is inward facing, and the first and second domains are both inward facing. When both domains are "inward facing", the chimeric protein would have an amino-terminal to carboxy-terminal configuration comprising an extracellular domain of a Type II transmembrane protein, a linker, and an extracellular domain of Type I transmembrane protein. In such configurations, it may be necessary for the chimeric protein to include extra "slack", as described elsewhere herein, to permit binding domains of the chimeric protein to one or both of its receptors/ligands.

In embodiments, the heterologous chimeric protein comprises: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L ligand, and (c) a linker linking the first domain and the second domain.

In embodiments, a heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a), and/or the second domain which comprises substantially the entire extracellular domain of CD40L. In embodiments, the first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a). In embodiments, the second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L ligand, and (c) a linker linking the first domain and the second domain.

In embodiments, a heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a), and/or the second domain which comprises substantially the entire extracellular domain of OX40L. In embodiments, the first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a). In embodiments, the second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding an LIGHT ligand, and (c) a linker linking the first domain and the second domain.

In embodiments, a heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a), and/or the second domain which comprises substantially the entire extracellular domain of LIGHT. In embodiments, the first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a). In embodiments, the second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, a chimeric protein used in methods of the present invention comprises the extracellular domain of human SIRPα(CD172a) which comprises the following amino acid sequence:

EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLT
KRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVVSGPAARATP
QHTVSFTCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTREDVHSQVIC
EVAHVTLQGDPLRGTANLSETIRVPPTLEVTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNV
SRTETASTVTENKDGTYNWMSWLLVNVSAHRDDVKLTCQVEHDGQPAVSKSHDLKVSAHPKEQ
GSNTAAENTGSNERNIY (SEQ ID NO: 57).

In embodiments, a chimeric protein used in methods of the present invention comprises a variant of the extracellular domain of SIRPα(CD172a). As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least

about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 57.

In embodiments, the variant of the extracellular domain of SIRPα(CD172a) has at least about 95% sequence identity with SEQ ID NO: 57

One of ordinary skill may select variants of the known amino acid sequence of SIRPα(CD172a) by consulting the literature, e.g. LEE, *et al.*, "Novel Structural Determinants of SIRPα that Mediate Binding of CD47," The Journal of Immunology, 179, 7741-7750, 2007 and HATHERLEY, *et al.*, "The Structure of the Macrophage Signal Regulatory Protein a (SIRPα) Inhibitory Receptor Reveals a Binding Face Reminiscent of That Used by T Cell Receptors," The Journal Of Biological Chemistry, Vol. 282, No. 19, pp. 14567-14575, 2007, each of which is incorporated by reference in its entirety.

In embodiments, a chimeric protein used in methods of the present invention comprises the extracellular domain of human CD40L which comprises the following amino acid sequence:

HRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFE
MQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGKQLTVKRQGLYYIYAQVTF
CSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGASVFVNVTD
PSQVSHGTGFTSFGLLKL (SEQ ID NO: 58).

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

In embodiments, the variant of the extracellular domain of CD40L has at least about 95% sequence identity with SEQ ID NO: 58

One of ordinary skill may select variants of the known amino acid sequence of CD40L by consulting the literature, *e.g.* An, *et al*. "Crystallographic and Mutational Analysis of the CD40-CD154 Complex and Its Implications for Receptor Activation", The Journal of Biological Chemistry 286, 11226-11235, which is incorporated by reference in its entirety.

In embodiments, a chimeric protein used in methods of the present invention comprises the extracellular domain of human OX40L which comprises the following amino acid sequence:

QVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVIINCDGFYLISLKGYFSQEVNISLHY
QKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLNVTTDNTSLDDFHVNGGELILIHQNPGEFCVL
(SEQ ID NO: 59).

In embodiments, a chimeric protein comprises a variant of the extracellular domain of OX40L. As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 59.

In embodiments, the variant of the extracellular domain of OX40L has at least about 95% sequence identity with SEQ ID NO: 59

One of ordinary skill may select variants of the known amino acid sequence of OX40L by consulting the literature, e.g., CROFT, et al., "The Significance of OX40 and OX40L to T cell Biology and Immune Disease," Immunol Rev., 229(1), PP. 173-191, 2009 and BAUM, et al., "Molecular characterization of murine and human OX40/0X40 ligand systems: identification of a human OX40 ligand as the HTL V-1-regulated protein gp34," The EMBO Journal, Vol. 13, No. 77, PP. 3992-4001, 1994, each of which is incorporated by reference in its entirety.

In embodiments, a chimeric protein used in methods of the present invention comprises the extracellular domain of human LIGHT which comprises the following amino acid sequence:

LQLHWRLGEMVTRLPDGPAGSWEQLIQERRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAF
LRGLSYHDGALVVTKAGYYYIYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCG
RATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLDERLVRLRDGTRSYFGAFMV (SEQ ID NO: 62).

In embodiments, a chimeric protein comprises a variant of the extracellular domain of LIGHT. As examples, the

variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 62.

In embodiments, the variant of the extracellular domain of LIGHT has at least about 95% sequence identity with SEQ ID NO: 62

One of ordinary skill may select variants of the known amino acid sequence of LIGHT by consulting the literature, e.g., Mauri, et al., "LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator." Immunity 8 (1), 21-30 (1998); Tamada et al., "LIGHT, a TNF-like molecule, costimulates T cell proliferation and is required for dendritic cell-mediated allogeneic T cell response." J. Immunol. 164 (8), 4105-4110 (2000); Liu et al., "Mechanistic basis for functional promiscuity in the TNF and TNF receptor superfamilies: structure of the LIGHT:DcR3 assembly" Structure 22 1252-62 (2014); Faustman et al., "Structural principles of tumor necrosis factor superfamily signaling." Sci Signal 11 (2018); Sudhamsu et al., "Dimerization of LTβR by LTα1β2 is necessary and sufficient for signal transduction" Proc. Natl. Acad. Sci. U.S.A. 110 19896-19901 (2013); Savvides et al., "Mechanisms of immunomodulation by mammalian and viral decoy receptors: insights from structures. Felix J, SN. Nat Rev Immunol 17 112-129 (2017)"; Ward-Kavanagh et al., "The TNF Receptor Superfamily in Co-stimulating and Co-inhibitory Responses." Immunity 44 1005-1019 (2016); and Wajant "Principles of antibody-mediated TNF receptor activation." Cell Death Differ 22 1727-1741 (2015), each of which is incorporated by reference in its entirety.

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

In embodiments, the amino acid mutations are amino acid substitutions, and may include conservative and/or non-conservative substitutions. "Conservative substitutions" may be made, for instance, based on similarity in polarity, charge, size, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the amino acid residues involved. The 20 naturally occurring amino acids can be grouped into the following six standard amino acid groups: (1) hydrophobic: Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr; Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. As used herein,

"conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed within the same group of the six standard amino acid groups shown above. For example, the exchange of Asp by Glu retains one negative charge in the so modified polypeptide. In addition, glycine and proline may be substituted for one another based on their ability to disrupt α-helices. As used herein, "non-conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed in a different group of the six standard amino acid groups (1) to (6) shown above.

In embodiments, the substitutions may also include non-classical amino acids (*e.g.,* selenocysteine, pyrrolysine, *N*-formylmethionine β-alanine, GABA and δ-Aminolevulinic acid, 4-aminobenzoic acid (PABA), D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosme, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β methyl amino acids, C α-methyl amino acids, N α-methyl amino acids, and amino acid analogs in general).

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

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

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

In embodiments, each extracellular domain (or variant thereof) of the chimeric protein binds to its cognate receptor or ligand with a K_D of about 1 nM to about 5 nM, for example, about 1 nM, about 1.5 nM, about 2 nM, about 2.5 nM, about 3 nM, about 3.5 nM, about 4 nM, about 4.5 nM, or about 5 nM. In embodiments, the chimeric protein binds to a cognate receptor or ligand with a K_D of about 5 nM to about 15 nM, for example, about 5 nM, about 5.5 nM, about 6 nM, about 6.5 nM, about 7 nM, about 7.5 nM, about 8 nM, about 8.5 nM, about 9 nM, about 9.5 nM, about 10 nM, about 10.5 nM, about 11 nM, about 11.5 nM, about 12 nM, about 12.5 nM, about 13 nM, about 13.5 nM, about 14 nM, about 14.5 nM, or about 15 nM.

In embodiments, each extracellular domain (or variant thereof) of the chimeric protein binds to its cognate receptor or ligand with a K_D of less than about 1 μM, about 900 nM, about 800 nM, about 700 nM, about 600 nM, about 500 nM, about 400 nM, about 300 nM, about 200 nM, about 150 nM, about 130 nM, about 100 nM, about 90 nM, about 80 nM, about 70 nM, about 60 nM, about 55 nM, about 50 nM, about 45 nM, about 40 nM, about 35 nM, about 30 nM, about 25 nM, about 20 nM, about 15 nM, about 10 nM, or about 5 nM, or about 1 nM (as measured, for example, by surface plasmon resonance or biolayer interferometry). In embodiments, the chimeric protein binds to human CSF1 with a K_D of less than about 1 nM, about 900 pM, about 800 pM, about 700 pM, about 600 pM, about 500 pM, about 400 pM, about 300 pM, about 200 pM, about 100 pM, about 90 pM, about 80 pM, about 70 pM, about 60 pM about 55 pM about

50 pM about 45 pM, about 40 pM, about 35 pM, about 30 pM, about 25 pM, about 20 pM, about 15 pM, or about 10 pM, or about 1 pM (as measured, for example, by surface plasmon resonance or biolayer interferometry).

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

In embodiments, the chimeric protein exhibits enhanced stability, high-avidity binding characteristics, prolonged off-rate for target binding and protein half-life relative to single-domain fusion protein or antibody controls.

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

Chimeric proteins of the present invention and/or chimeric proteins used in methods of the present invention have a first domain which is sterically capable of binding its ligand/receptor and/or a second domain which is sterically capable of binding its ligand/receptor. This means that there is sufficient overall flexibility in the chimeric protein and/or physical distance between an extracellular domain (or a portion thereof) and the rest of the chimeric protein such that the ligand/receptor binding domain of the extracellular domain is not sterically hindered from binding its ligand/receptor. This flexibility and/or physical distance (which is herein referred to as "slack") may be normally present in the extracellular domain(s), normally present in the linker, and/or normally present in the chimeric protein (as a whole). Alternately, or additionally, the chimeric protein may be modified by including one or more additional amino acid sequences (*e.g.,* the joining linkers described below) or synthetic linkers (*e.g.,* a polyethylene glycol (PEG) linker) which provide additional slack needed to avoid steric hindrance.

Linkers

In embodiments, the chimeric protein used in a method of the present invention comprises a linker.

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

Importantly, *inter alia*, stabilization in a linker region including one or more disulfide bonds provides for improved chimeric proteins that can maintain a stable and producible multimeric state.

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

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

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

In embodiments, the linker is flexible.

In embodiments, the linker is rigid.

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

In embodiments, the linker comprises a hinge region of an antibody (*e.g.,* of IgG, IgA, IgD, and IgE, inclusive of subclasses (*e.g.,* IgG1, IgG2, IgG3, and IgG4, and IgA1, and IgA2)). The hinge region, found in IgG, IgA, IgD, and IgE class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses. For example, the length and flexibility of the hinge region varies among the IgG subclasses.

The hinge region of IgG1 encompasses amino acids 216-231 and, because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, is relatively short, and contains a rigid poly-proline double helix, stabilized by extra inter-heavy chain disulfide bridges. These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), containing 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3, the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge regions reportedly decreases in the order IgG3>IgG1>IgG4>IgG2. In embodiments, the linker may be derived from human IgG4 and contain one or more mutations to enhance dimerization (including S228P) or FcRn binding.

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. *See* Shin *et al.*, 1992 *Immunological Reviews* 130:87. The upper hinge region includes amino acids from the carboxyl end of C_{H1} to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end of the C_{H2} domain and includes residues in C_{H2}. *Id.* The core hinge region of wild-type human IgG1 contains the sequence CPPC (SEQ ID NO: 24) which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. In embodiments, the present linker comprises, one, or two, or three of the upper hinge region, the core region, and the lower hinge region of any antibody (*e.g.,* of IgG, IgA, IgD, and IgE, inclusive of subclasses (*e.g.,* IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)). The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains five glycosylation sites within a 17-amino-acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin. In embodiments, the linker of the present invention comprises one or more glycosylation sites.

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

In a chimeric protein used in a method of the present invention, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from a human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence

of any one of SEQ ID NO: 1 to SEQ ID NO: 3, *e.g.,* at least 95% identical to the amino acid sequence of SEQ ID NO: 2. In embodiments, the linker comprises one or more joining linkers, such joining linkers independently selected from SEQ ID NO: 4 to SEQ ID NO: 50 (or a variant thereof). In embodiments, the linker comprises two or more joining linkers each joining linker independently selected from SEQ ID NO: 4 to SEQ ID NO: 50 (or a variant thereof); wherein one joining linker is N terminal to the hinge-CH2-CH3 Fc domain and another joining linker is C terminal to the hinge-CH2-CH3 Fc domain.

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

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

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

In embodiments, the linker comprises an IgG constant region that contains one or more mutations at amino acid residues 250, 253, 307, 310, 380, 428, 433, 434, and 435 (in accordance with Kabat numbering, as in as in Kabat, *et al*., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) expressly incorporated herein by reference). Illustrative mutations include T250Q, M428L, T307A, E380A, I253A, H310A, M428L, H433K, N434A, N434F, N434S, and H435A. In embodiments, the IgG constant region comprises a M428L/N434S mutation or LS mutation. In embodiments, the IgG constant region comprises a T250Q/M428L mutation or QL mutation. In embodiments, the IgG constant region comprises an N434A mutation. In embodiments, the IgG constant region comprises a T307A/E380A/N434A mutation or AAA mutation. In embodiments, the IgG constant region comprises an I253A/H310A/H435A mutation or IHH mutation. In embodiments, the IgG constant region comprises a H433K/N434F mutation. In embodiments, the IgG constant region comprises a M252Y/S254T/T256E and a H433K/N434F mutation in combination.

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

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

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

In embodiments, the Fc domain in a linker has the amino acid sequence of SEQ ID NO: 1 (see **Table 1**, below), or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto. In embodiments, mutations are made to SEQ ID NO: 1 to increase stability and/or half-life. For instance, in embodiments, the Fc domain in a linker comprises the amino acid sequence of SEQ ID NO: 2 (see **Table 1**, below), or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto. For instance, in embodiments, the Fc domain in a linker comprises the amino acid sequence of SEQ ID NO: 3 (see **Table 1**, below), or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto.

Further, one or more joining linkers may be employed to connect an Fc domain in a linker (*e.g.,* one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or at least 90%, or 93%, or 95%, or 97%, or 98%, or 99% identity thereto) and the extracellular domains. For example, any one of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or variants thereof may connect an extracellular domain as disclosed herein and an Fc domain in a linker as disclosed herein. Optionally, any one of SEQ ID NO: 4 to SEQ ID NO: 50, or variants thereof are located between an extracellular domain as disclosed herein and an Fc domain as disclosed herein.

In embodiments, the chimeric proteins used in methods of the present invention may comprise variants of the joining linkers disclosed in **Table 1**, below. For instance, a linker may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the amino acid sequence of any one of SEQ ID NO: 4 to SEQ ID NO: 50.

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

Without wishing to be bound by theory, including a linker comprising at least a part of an Fc domain in a chimeric protein, helps avoid formation of insoluble and, likely, non-functional protein concatenated oligomers and/or aggregates. This is in part due to the presence of cysteines in the Fc domain which are capable of forming disulfide bonds between chimeric proteins.

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

In embodiments, the first and/or second joining linkers are independently selected from the amino acid sequences of SEQ ID NO: 4 to SEQ ID NO: 50 and are provided in **Table 1** below:

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

SEQ ID NO.	Sequence
1	APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSSWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
2	APEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTTPHSDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSSWQEGNVFSCSVLHEALHNH YTQKSLSLSLGK
3	APEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNH YTQKSLSLSLGK
4	SKYGPPCPSCP
5	SKYGPPCPPCP
6	SKYGPP
7	IEGRMD
8	GGGVPRDCG
9	IEGRMDGGGGAGGGG
10	GGGSGGGS
11	GGGSGGGGSGGG
12	EGKSSGSGSESKST
13	GGSG
14	GGSGGGSGGGSG
15	EAAAKEAAAKEAAAK
16	EAAAREAAAREAAAREAAAR
17	GGGGSGGGGSGGGGSAS
18	GGGGAGGGG
19	GS or GGS or LE
20	GSGSGS
21	GSGSGSGSGS
22	GGGGSAS
23	APAPAPAPAPAPAPAPAPAP
24	CPPC
25	GGGGS
26	GGGGSGGGGS
27	GGGGSGGGGSGGGGS
28	GGGGSGGGGSGGGGSGGGGS
29	GGGGSGGGGSGGGGSGGGGSGGGGS
30	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
31	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
32	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
33	GGSGGSGGGGSGGGGS
34	GGGGGGGG
35	GGGGGG
36	EAAAK

37	EAAAKEAAAK
38	EAAAKEAAAKEAAAK
39	AEAAAKEAAAKA
40	AEAAAKEAAAKEAAAKA
41	AEAAAKEAAAKEAAAKEAAAKA
42	AEAAAKEAAAKEAAAKEAAAKEAAAKA
43	AEAAAKEAAAKEAAAKEAAAKALEAEAAAKEAAAKEAAAKEAAAKA
44	PAPAP
45	KESGSVSSEQLAQFRSLD
46	GSAGSAAGSGEF
47	GGGSE
48	GSESG
49	GSEGS
50	GEGGSGEGSSGEGSSSEGGGSEGGGSEGGGSEGGS

In embodiments, the joining linker substantially comprises glycine and serine residues (*e.g.,* about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 97%, or about 98%, or about 99%, or about 100% glycines and serines). For example, in embodiments, the joining linker is $(Gly_4Ser)_n$, where n is from about 1 to about 8, *e.g.,* 1, 2, 3, 4, 5, 6, 7, or 8 (SEQ ID NO: 25 to SEQ ID NO: 32, respectively). In embodiments, the joining linker sequence is GGSGGSGGGGSGGGGS (SEQ ID NO: 33). Additional illustrative joining linkers include, but are not limited to, linkers having the sequence LE, $(EAAAK)_n$ (n=1-3) (SEQ ID NO: 36 to SEQ ID NO: 38), $A(EAAAK)_nA$ (n = 2-5) (SEQ ID NO: 39 to SEQ ID NO: 42), $A(EAAAK)_4ALEA(EAAAK)_4A$ (SEQ ID NO: 43), PAPAP (SEQ ID NO: 44), KESGSVSSEQLAQFRSLD (SEQ ID NO: 45), GSAGSAAGSGEF (SEQ ID NO: 46), and $(XP)_n$, with X designating any amino acid, *e.g.,* Ala, Lys, or Glu. In embodiments, the joining linker is GGS. In embodiments, a joining linker has the sequence $(Gly)_n$ where n is any number from 1 to 100, for example: $(Gly)_8$ (SEQ ID NO: 34) and $(Gly)_6$ (SEQ ID NO: 35).

In embodiments, the joining linker is one or more of GGGSE (SEQ ID NO: 47), GSESG (SEQ ID NO: 48), GSEGS (SEQ ID NO: 49), GEGGSGEGSSGEGSSSEGGGSEGGGSEGGGSEGGS (SEQ ID NO: 50), and a joining linker of randomly placed G, S, and E every 4 amino acid intervals.

In embodiments, where a chimeric protein used in a method of the present invention comprises an extracellular domain (ECD) of a first transmembrane protein, one joining linker preceding an Fc domain, a second joining linker following the Fc domain, and an ECD of second transmembrane protein, the chimeric protein may comprise the following structure:

ECD – Joining Linker 1 – Fc Domain – Joining Linker 2 – ECD.

The combination of a first joining linker, an Fc Domain linker, and a second joining linker is referend to herein as a "modular linker". In embodiments, a chimeric protein used in a method of the present invention comprises a modular linker as shown in **Table 2:**

Table 2: Illustrative modular linkers

Joining Linker 1	Fc	Joining Linker 2	Modular Linker = Joining Linker 1 + Fc + Joining Linker 2
SKYGPPCPSCP (SEQ ID NO: 4)	APEFLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLSGKEYKC KVSSKGLPSSIEKTISNATGQPRE PQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSRLTVDKS SWQEGNVFSCSVMHEALHNHYT QKSLSLSLGK (SEQ ID NO: 1)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPSCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQ DWLSGKEYKCKVSSKGLPSSIEK TISNATGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSSWQEGNVFSC SVMHEALHNHYTQKSLSLSLGKIE GRMD (SEQ ID NO: 51)
SKYGPPCPSCP (SEQ ID NO: 4)	APEFLGGPSVFLFPPKPKDQLMIS RTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNS TYRVVSVLTTPHSDWLSGKEYKC KVSSKGLPSSIEKTISNATGQPRE PQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSRLTVDKS SWQEGNVFSCSVLHEALHNHYT QKSLSLSLGK (SEQ ID NO: 2)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPSCPAPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTTPH SDWLSGKEYKCKVSSKGLPSSIE KTISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSSWQEGNVFS CSVLHEALHNHYTQKSLSLSLGKI EGRMD (SEQ ID NO: 52)
SKYGPPCPSCP (SEQ ID NO: 4)	APEFLGGPSVFLFPPKPKDQLMIS RTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLSGKEYKC KVSSKGLPSSIEKTISNATGQPRE PQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVLHEALHNHYT QKSLSLSLGK (SEQ ID NO: 3)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPSCPAPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLH QDWLSGKEYKCKVSSKGLPSSIE KTISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFS CSVLHEALHNHYTQKSLSLSLGKI EGRMD (SEQ ID NO: 53)
SKYGPPCPPCP (SEQ ID NO: 5)	APEFLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLSGKEYKC KVSSKGLPSSIEKTISNATGQPRE PQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSRLTVDKS SWQEGNVFSCSVMHEALHNHYT QKSLSLSLGK (SEQ ID NO: 1)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQ DWLSGKEYKCKVSSKGLPSSIEK TISNATGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSSWQEGNVFSC SVMHEALHNHYTQKSLSLSLGKIE GRMD (SEQ ID NO: 54)
SKYGPPCPPCP (SEQ ID NO: 5)	APEFLGGPSVFLFPPKPKDQLMIS RTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNS	IEGRMD (SEQ ID NO: 7)	SKYGPPCPPCPAPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNA

	TYRVVSVLTTPHSDWLSGKEYKC KVSSKGLPSSIEKTISNATGQPRE PQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSRLTVDKS SWQEGNVFSCSVLHEALHNHYT QKSLSLSLGK (SEQ ID NO: 2)		KTKPREEQFNSTYRVVSVLTTPH SDWLSGKEYKCKVSSKGLPSSIE KTISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSSWQEGNVFS CSVLHEALHNHYTQKSLSLSLGKI EGRMD (SEQ ID NO: 55)
SKYGPPCPPCP (SEQ ID NO: 5)	APEFLGGPSVFLFPPKPKDQLMIS RTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLSGKEYKC KVSSKGLPSSIEKTISNATGQPRE PQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVLHEALHNHYT QKSLSLSLGK (SEQ ID NO: 3)	IEGRMD (SEQ ID NO: 7)	SKYGPPCPPCPAPEFLGGPSVFL FPPKPKDQLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLH QDWLSGKEYKCKVSSKGLPSSIE KTISNATGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFS CSVLHEALHNHYTQKSLSLSLGKI EGRMD (SEQ ID NO: 56)

In embodiments, the chimeric proteins used in methods of the present invention may comprise variants of the modular linkers disclosed in **Table 2**, above. For instance, a linker may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with the amino acid sequence of any one of SEQ ID NO: 51 to SEQ ID NO: 56.

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

Table 3: Characteristics of illustrative joining linkers

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

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

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

In embodiments, a chimeric protein used in a method of the present invention comprises only one joining linkers.

In embodiments, a chimeric protein used in a method of the present invention lacks joining linkers.

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

In embodiments, a chimeric protein has a first domain which is sterically capable of binding its ligand/receptor and/or the second domain which is sterically capable of binding its ligand/receptor. Thus, there is enough overall flexibility in the chimeric protein and/or physical distance between an extracellular domain (or portion thereof) and the rest of the chimeric protein such that the ligand/receptor binding domain of the extracellular domain is not sterically hindered from binding its ligand/receptor. This flexibility and/or physical distance (which is referred to as “slack”) may be normally present in the extracellular domain(s), normally present in the linker, and/or normally present in the chimeric protein (as a whole). Alternately, or additionally, an amino acid sequence (for example) may be added to one or more extracellular domains and/or to the linker to provide the slack needed to avoid steric hindrance. Any amino acid sequence that provides slack may be added. In embodiments, the added amino acid sequence comprises the sequence $(Gly)_n$ where n is any number from 1 to 100. Additional examples of addable amino acid sequence include the joining linkers described in **Table 1** and **Table 3**. In embodiments, a polyethylene glycol (PEG) linker may be added between an extracellular domain and a linker to provide the slack needed to avoid steric hindrance. Such PEG linkers are well known in the art.

In embodiments, a heterologous chimeric protein comprises a first domain comprising a portion of SIRPα(CD172a), a second domain comprising a portion of CD40L, and a linker. In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence. In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain, e.g., from an IgG1 or from IgG4, including human IgG1 or IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. Thus, in embodiments, when a heterologous chimeric protein used in a method of the present invention comprises the extracellular domain of SIRPα(CD172a) (or a variant thereof), a linker comprising a hinge-CH2-CH3 Fc domain, and the extracellular domain of CD40L (or a variant thereof), it may be referred to herein as "SIRPα(CD172a)-Fc-CD40L".

In embodiments, a SIRPα(CD172a)-Fc-CD40L chimeric protein of the present invention and/or a chimeric protein used in methods of the present invention has the following amino acid sequence:

EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTKR
NNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVVSGPAARATPQHTVSF
TCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTREDVHSQVICEVAHVTLQG
DPLRGTANLSETIRVPPTLEVTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSRTETASTVTEN
KDGTYNWMSWLLVNVSAHRDDVKLTCQVEHDGQPAVSKSHDLKVSAHPKEQGSNTAAENTGSNERN
IYSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVLHEALHNHYTQKSLSLSLGKIEGRMDHRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLN
CEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNL
VTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQ
SIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKL (SEQ IN NO: 60)

In embodiments, a chimeric protein comprises a variant of a SIRPα(CD172a)-Fc-CD40L chimeric protein. As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about

95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 60.

In embodiments, a heterologous chimeric protein comprises a first domain comprising a portion of SIRPα(CD172a), a second domain comprising a portion of OX40L, and a linker. In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence. In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain, e.g., from an IgG1 or from IgG4, including human IgG1 or IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. Thus, in embodiments, when a heterologous chimeric protein used in a method of the present invention comprises the extracellular domain of SIRPα(CD172a) (or a variant thereof), a linker comprising a hinge-CH2-CH3 Fc domain, and the extracellular domain of OX40L (or a variant thereof), it may be referred to herein as "SIRPα(CD172a)-Fc-OX40L".

In embodiments, a SIRPα(CD172a)-Fc-OX40L chimeric protein of the present invention and/or a chimeric protein used in methods of the present invention has the following amino acid sequence:

EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTKR
NNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVVSGPAARATPQHTVSF
TCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTREDVHSQVICEVAHVTLQG
DPLRGTANLSETIRVPPTLEVTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSRTETASTVTEN
KDGTYNWMSWLLVNVSAHRDDVKLTCQVEHDGQPAVSKSHDLKVSAHPKEQGSNTAAENTGSNERN
IYSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVLHEALHNHYTQKSLSLSLGKIEGRMDQVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKV
QNNSVIINCDGFYLISLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLNVTTDNT
SLDDFHVNGGELILIHQNPGEFCVL(SEQ IN NO: 61)

In embodiments, a chimeric protein comprises a variant of a SIRPα(CD172a)-Fc-OX40L chimeric protein. As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least

about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 61.

In embodiments, a heterologous chimeric protein comprises a first domain comprising a portion of SIRPα(CD172a), a second domain comprising a portion of LIGHT, and a linker. In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence. In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain, e.g., from an IgG1 or from IgG4, including human IgG1 or IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. Thus, in embodiments, when a heterologous chimeric protein used in a method of the present invention comprises the extracellular domain of SIRPα(CD172a) (or a variant thereof), a linker comprising a hinge-CH2-CH3 Fc domain, and the extracellular domain of LIGHT (or a variant thereof), it may be referred to herein as "SIRPα(CD172a)-Fc-LIGHT".

In embodiments, a SIRPα(CD172a)-Fc-LIGHT chimeric protein of the present invention and/or a chimeric protein used in methods of the present invention has the following amino acid sequence:

EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTKR
NNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVVSGPAARATPQHTVSF
TCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTREDVHSQVICEVAHVTLQG
DPLRGTANLSETIRVPPTLEVTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSRTETASTVTEN
KDGTYNWMSWLLVNVSAHRDDVKLTCQVEHDGQPAVSKSHDLKVSAHPKEQGSNTAAENTGSNERN
IYSKYGPPCPPCPAPEFLGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYKCKVSSKGLPSSIEKTISNATGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVLHEALHNHYTQKSLSLSLGKIEGRMDLQLHWRLGEMVTRLPDGPAGSWEQLIQERRSHEVN
PAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYYIYSKVQLGGVGCPLGLASTI
THGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLDERLVRLRD
GTRSYFGAFMV (SEQ ID NO: 63)

In embodiments, a chimeric protein comprises a variant of a SIRPα(CD172a)-Fc-LIGHT chimeric protein. As examples, the variant may have at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%,

or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 63.

Diseases, Methods of Treatment, and Mechanisms of Action

The methods comprise steps of administering to a subject in need thereof (either simultaneously or sequentially) an effective amount of at least one antibody directed to an immune checkpoint molecule; a stimulator of interferon genes (STING) agonist; and/or one or more chimeric proteins, in which each chimeric protein is capable of blocking immune inhibitory signals and/or stimulating immune activating signals.

It is often desirable to disrupt, block, reduce, inhibit, and/or sequester the transmission of immune inhibitory signals and, simultaneously or contemporaneously, enhance, increase, and/or stimulate the transmission of an immune stimulatory signal to an anti-cancer immune cell, to boost an immune response, for instance to enhance a patient's anti-tumor immune response.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of, or can be used in methods comprising, modulating the amplitude of an immune response, e.g., modulating the level of effector output.

In embodiments, e.g. when used for the treatment of cancer, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention alter the extent of immune stimulation as compared to immune inhibition to increase the amplitude of a T cell response, including, without limitation, stimulating increased levels of cytokine production, proliferation or target killing potential. In embodiments, the patient's T cells are activated and/or stimulated by the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention, with the activated T cells being capable of dividing and/or secreting cytokines.

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

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

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

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

Representative cancers and/or tumors of the present invention include, but are not limited to, a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention treat a subject that has a treatment-refractory cancer. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention treat a subject that is refractory to one or more immune-modulating agents. For example, in embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention treat a subject that presents no response to treatment, or even progress, after 12 weeks or so of treatment. For instance, in embodiments, the subject is refractory to a PD-1 and/or PD-L1 and/or PD-L2 agent, including, for example, nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and/or MPDL328OA (ROCHE)-refractory patients. For instance, in embodiments, the subject is refractory to an anti-CTLA-4 agent, *e.g.,* ipilimumab (YERVOY)-refractory patients (*e.g.,* melanoma patients). Accordingly, in embodiments the present invention provides methods of cancer treatment that rescue patients that are non-responsive to various therapies, including monotherapy of one or more immune-modulating agents.

In embodiments, the present invention provides antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins which target a cell or tissue within the tumor microenvironment. In embodiments, the cell or tissue within the tumor microenvironment expresses one or more targets or binding partners of the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention. The tumor microenvironment refers to the cellular milieu, including cells, secreted proteins, physiological small molecules, and blood vessels in which the tumor exists. In embodiments, the cells or tissue within the tumor microenvironment are one or more of: tumor vasculature; tumor-infiltrating lymphocytes; fibroblast reticular cells; endothelial progenitor cells (EPC); cancer-associated fibroblasts; pericytes; other stromal cells; components of the extracellular matrix (ECM); dendritic cells; antigen presenting cells; T-cells; regulatory T cells; macrophages; neutrophils; and other immune cells located proximal to a tumor. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention targets a cancer cell. In embodiments, the cancer cell expresses one or more of targets or binding partners of the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention.

In embodiments, the present methods provide treatment with the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins in a patient who is refractory to an additional agent, such “additional agents” being disclosed elsewhere herein, inclusive, without limitation, of the various chemotherapeutic agents disclosed herein.

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

In embodiments, an immune stimulatory signal refers to a signal that enhances an immune response. For example, in the context of oncology, such signals may enhance antitumor immunity. For instance, without limitation, immune stimulatory signal may be identified by directly stimulating proliferation, cytokine production, killing activity, or phagocytic activity of leukocytes. Specific examples include direct stimulation of TNF superfamily receptors such as OX40, LTbR, CD27, CD30,4-1BB or TNFRSF25 using either receptor agonist antibodies or using a chimeric protein comprising the ligands for such receptors (OX40L, LIGHT, CD70, CD30L, 4-1BBL, TL1A, respectively). Stimulation from any one of these receptors may directly stimulate the proliferation and cytokine production of individual T cell subsets. Another example includes direct stimulation of an immune inhibitory cell with through a receptor that inhibits the activity of such an immune suppressor cell. This would include, for example, stimulation of CD4+FoxP3+ regulatory T cells with a GITR agonist antibody or GITRL containing chimeric protein, which would reduce the ability of those regulatory T cells to suppress the proliferation of conventional CD4+ or CD8+ T cells. In another example, this would include stimulation of CD40 on the surface of an antigen-presenting cell using a CD40 agonist antibody or a chimeric protein comprising CD40L, causing activation of antigen presenting cells including enhanced ability of those cells to present antigen in the context of appropriate native costimulatory molecules, including those in the B7 or TNF superfamily. In another example, this would include stimulation of LTBR on the surface of a lymphoid or stromal cell using a LIGHT containing chimeric protein, causing activation of the lymphoid cell and/or production of pro-inflammatory cytokines or chemokines to further stimulate an immune response, optionally within a tumor.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins are capable of, or find use in methods involving, enhancing, restoring, promoting and/or stimulating immune modulation. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention described herein, restore, promote and/or stimulate the activity or activation of one or more immune cells against tumor cells including, but not limited to: T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, anti-tumor macrophages (*e.g.* M1 macrophages), B cells, and dendritic cells. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention enhance, restore, promote and/or stimulate the activity and/or activation of T cells, including, by way of a non-limiting example, activating and/or stimulating one or more T-cell intrinsic signals, including a pro-survival signal; an autocrine or paracrine growth signal; a p38 MAPK-, ERK-, STAT-, JAK-, AKT- or PI3K-mediated signal; an anti-apoptotic signal; and/or a signal promoting and/or necessary for one or more of: pro-inflammatory cytokine production or T cell migration or T cell tumor infiltration.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of, or find use in methods involving, causing an increase of one or more of T cells (including without limitation cytotoxic T lymphocytes, T helper cells, natural killer T (NKT) cells), B cells, natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells, monocytes, and macrophages (*e.g.,* one or more of M1 and M2) into a tumor or the tumor microenvironment. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention enhance recognition of tumor antigens by CD8+ T cells, particularly those T cells that have infiltrated into the tumor microenvironment. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention induce CD19 expression and/or increases the number of CD19 positive cells (*e.g.,* CD19 positive B cells). In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention induce IL-15Rα expression and/or increases the number of IL-15Rα positive cells (*e.g.,* IL-15Rα positive dendritic cells).

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of, or find use in methods involving, inhibiting and/or causing a decrease in immunosuppressive cells (*e.g.,* myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), tumor associated neutrophils (TANs), M2 macrophages, and tumor associated macrophages (TAMs)), and particularly within the tumor and/or tumor microenvironment (TME). In embodiments, the present therapies may alter the ratio of M1 versus M2 macrophages in the tumor site and/or TME to favor M1 macrophages.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are able to increase the serum levels of various cytokines or chemokines including, but not limited to, one or more of IFNγ, TNFα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-13, IL-15, IL-17A, IL-17F, IL-22, CCL2, CCL3, CCL4, CXCL8, CXCL9, CXCL10, CXCL11 and CXCL12. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of enhancing IL-2, IL-4, IL-5, IL-10, IL-13, IL-17A, IL-22, TNFα or IFNγ in the serum of a treated subject. In embodiments, administration of the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention is capable of enhancing TNFα secretion. In a specific embodiment, administration of the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention is capable of enhancing superantigen mediated TNFα secretion by leukocytes. Detection of such a cytokine response may provide a method to determine the optimal dosing regimen for the indicated antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention.

The antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of increasing or preventing a decrease in a sub-population of CD4+ and/or CD8+ T cells.

The antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of enhancing tumor-killing activity by T cells.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention inhibit, block and/or reduce cell death of an anti-tumor CD8+ and/or CD4+ T cell; or stimulate, induce, and/or increase cell death of a pro-tumor T cell. T cell exhaustion is a state of T cell dysfunction characterized by progressive loss of proliferative and effector functions, culminating in clonal deletion. Accordingly, a pro-tumor T cell refers to a state of T cell dysfunction that arises during many chronic infections, inflammatory diseases, and cancer. This dysfunction is defined by poor proliferative and/or effector functions, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. Illustrative pro-tumor T cells include, but are not limited to, Tregs, CD4+ and/or CD8+ T cells expressing one or more checkpoint inhibitory receptors, Th2 cells and Th17 cells. Checkpoint inhibitory receptors refer to receptors expressed on immune cells that prevent or inhibit uncontrolled immune responses. In contrast, an anti-tumor CD8+ and/or CD4+ T cell refers to T cells that can mount an immune response to a tumor.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of, and can be used in methods comprising, increasing a ratio of effector T cells to regulatory T cells. Illustrative effector T cells include $ICOS^+$ effector T cells; cytotoxic T cells (*e.g.,* αβ TCR, $CD3^+$, $CD8^+$, $CD45RO^+$); $CD4^+$ effector T cells (*e.g.,* αβ TCR, $CD3^+$, $CD4^+$, $CCR7^+$, CD62Lhi, $IL\text{-}7R/CD127^+$); $CD8^+$ effector T cells (*e.g.,* αβ TCR, $CD3^+$, $CD8^+$, $CCR7^+$, CD62Lhi, $IL\text{-}7R/CD127^+$); effector memory T cells (*e.g.,* CD62Llow, $CD44^+$, TCR, $CD3^+$, $IL\text{-}7R/CD127^+$, $IL\text{-}15R^+$, CCR7low); central memory T cells (*e.g.,* $CCR7^+$, $CD62L^+$, $CD27^+$; or CCR7hi, $CD44^+$, CD62Lhi, TCR, $CD3^+$, IL-7R/$CD127^+$, $IL\text{-}15R^+$); $CD62L^+$ effector T cells; $CD8^+$ effector memory T cells (TEM) including early effector memory T cells ($CD27^+$ $CD62L^-$) and late effector memory T cells ($CD27^-$ $CD62L^-$) (TemE and TemL, respectively); $CD127(^+)CD25$(low/-) effector T cells; $CD127(^-)CD25(^-)$ effector T cells; $CD8^+$ stem cell memory effector cells (TSCM) (*e.g.,* CD44(low)CD62L(high)CD122(high)$sca(^+)$); TH1 effector T-cells (*e.g.,* $CXCR3^+$, $CXCR6^+$ and $CCR5^+$; or αβ TCR, $CD3^+$, $CD4^+$, $IL\text{-}12R^+$, $IFN\gamma R^+$, $CXCR3^+$), TH2 effector T cells (*e.g.,* $CCR3^+$, $CCR4^+$ and $CCR8^+$; or αβ TCR, $CD3^+$, $CD4^+$, $IL\text{-}4R^+$, $IL\text{-}33R^+$, $CCR4^+$, $IL\text{-}17RB^+$, $CRTH2^+$); TH9 effector T cells (*e.g.,* αβ TCR, $CD3^+$, $CD4^+$); TH17 effector T cells (*e.g.,* αβ TCR, $CD3^+$, $CD4^+$, $IL\text{-}23R^+$, $CCR6^+$, $IL\text{-}1R^+$); $CD4^+CD45RO^+CCR7^+$ effector T cells, $CD4^+CD45RO^+CCR7(^-)$ effector T cells; and effector T cells secreting IL-2, IL-4 and/or IFN-γ. Illustrative regulatory T cells include $ICOS^+$ regulatory T cells, $CD4^+CD25^+FOXP3^+$ regulatory T cells, $CD4^+CD25^+$ regulatory T cells, $CD4^+CD25^-$ regulatory T cells, $CD4^+$CD25high regulatory T cells, $TIM\text{-}3^+PD\text{-}1^+$

regulatory T cells, lymphocyte activation gene-3 (LAG-3)$^+$ regulatory T cells, CTLA-4/CD152$^+$ regulatory T cells, neuropilin-1 (Nrp-1)$^+$ regulatory T cells, CCR4$^+$CCR8$^+$ regulatory T cells, CD62L (L-selectin)$^+$ regulatory T cells, CD45RBlow regulatory T cells, CD127low regulatory T cells, LRRC32/GARP$^+$ regulatory T cells, CD39$^+$ regulatory T cells, GITR$^+$ regulatory T cells, LAP$^+$ regulatory T cells, 1B11$^+$ regulatory T cells, BTLA$^+$ regulatory T cells, type 1 regulatory T cells (Tr1 cells),T helper type 3 (Th3) cells, regulatory cell of natural killer T cell phenotype (NKTregs), CD8$^+$ regulatory T cells, CD8$^+$CD28$^-$ regulatory T cells and/or regulatory T-cells secreting IL-10, IL-35, TGF-β, TNF-α, Galectin-1, IFN-γ and/or MCP1.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention cause an increase in effector T cells (*e.g.,* CD4+CD25- T cells).

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention cause a decrease in regulatory T cells (*e.g.,* CD4+CD25+ T cells).

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention generate a memory response which may be capable of preventing relapse or protecting the animal from a recurrence and/or preventing, or reducing the likelihood of, metastasis. Thus, an animal treated with the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention is later able to attack tumor cells and/or prevent development of tumors when rechallenged after an initial treatment with the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention. Accordingly, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention stimulate both active tumor destruction and also immune recognition of tumor antigens, which are essential in programming a memory response capable of preventing relapse.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of causing activation of antigen presenting cells. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable enhancing the ability of antigen presenting cells to present antigen.

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

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

The chimeric proteins used in methods of the present invention are capable of forming a stable synapse between cells. The stable synapse of cells promoted by the chimeric proteins (*e.g.,* between cells bearing negative signals) provides spatial orientation to favor tumor reduction - such as positioning the T cells to attack tumor cells and/or sterically preventing the tumor cell from delivering negative signals, including negative signals beyond those masked by the chimeric proteins. In embodiments, this provides longer on-target (*e.g.,* intra-tumoral) half-life ($t_{1/2}$) as compared to serum $t_{1/2}$ of the chimeric proteins. Such properties could have the combined advantage of reducing off-target toxicities associated with systemic distribution of the chimeric proteins.

In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention are capable of providing a sustained immunomodulatory effect.

The antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention provide synergistic therapeutic effects (*e.g.,* anti-tumor effects) as it allows for improved site-specific interplay of two immunotherapy agents. In embodiments, the antibodies directed to immune checkpoint molecules, STING agonists, and/or chimeric proteins used in methods of the present invention provide the potential for reducing off-site and/or systemic toxicity.

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

In embodiments, the chimeric proteins used in methods of the present invention provide reduced side effects, *e.g.,* GI complications, relative to current immunotherapies, *e.g.,* antibodies directed to ligand(s)/receptor(s) targeted by the

extracellular domains of the chimeric proteins used in methods of the present invention used in methods of the present invention. Illustrative GI complications include abdominal pain, appetite loss, autoimmune effects, constipation, cramping, dehydration, diarrhea, eating problems, fatigue, flatulence, fluid in the abdomen or ascites, gastrointestinal (GI) dysbiosis, GI mucositis, inflammatory bowel disease, irritable bowel syndrome (IBS-D and IBS-C), nausea, pain, stool or urine changes, ulcerative colitis, vomiting, weight gain from retaining fluid, and/or weakness.

Methods of treatment

In various aspects, the present invention provides compositions and methods that are useful for cancer immunotherapy. For instance, the present invention, in part, relates to methods for treating cancer comprising administering (either simultaneously or sequentially) two chimeric proteins in which each chimeric protein is capable of blocking immune inhibitory signals and/or stimulating immune activating signals.

In embodiments, the chimeric proteins of the present invention and/or chimeric proteins used in methods of the present invention eliminate or reduce side effects associated with disrupting the SIRP1α/CD47 signaling axis. In embodiments, the present chimeric proteins or methods utilizing the same eliminate or reduce hematological adverse effects. In embodiments, the present chimeric proteins or methods utilizing the same eliminate or reduce the extent of reductions in the number of circulating red blood cells and platelets, hemolysis, hemagglutination, thrombocytopenia, and/or anemia. In embodiments, the present chimeric proteins or methods utilizing the same demonstrate comparatively less hematological adverse effects than an anti-CD47 antibody.

An aspect of the present invention is a method for treating a cancer in a subject in need thereof. The method comprises steps of providing the subject a first pharmaceutical composition and providing the subject a second pharmaceutical composition. The first pharmaceutical composition comprises a heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. The second pharmaceutical composition comprises an antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), or human epidermal growth factor receptor 2 (Her2), or and capable of, respectively, inhibiting the interaction of CD20, EGFR, or Her2, with one or more of its ligands.

In embodiments, the first pharmaceutical composition and the second pharmaceutical composition are provided simultaneously, the first pharmaceutical composition is provided after the second pharmaceutical composition is provided, or the first pharmaceutical composition is provided before the second pharmaceutical composition is provided.

In embodiments, the dose of the first pharmaceutical composition is less than the dose of the first pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the second pharmaceutical composition.

In embodiments, the dose of the second pharmaceutical composition provided is less than the dose of the second pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the second pharmaceutical composition.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody capable of binding CD20 is selected from rituximab, obinutuzumab, ofatumumab, ocrelizumab, ocaratuzumab, and veltuzumab. In embodiments, the antibody capable of binding CD20 is rituximab.

In embodiments, the antibody capable of binding EGFR is selected from cetuximab, ABP 494 (Actavis), CT-P15 (Celltrion), STI-001 (Sorrento), panitumumab, necitumumab, nimotuzumab, matuzumab, and chimeric 806 (ch806). In embodiments, the antibody capable of binding EGFR is cetuximab.

In embodiments, the antibody capable of binding HER2 is selected from trastuzumab, trastuzumab deruxtecan, ado-trastuzumab emtansine (T-DM1), trastuzumab-pkrb, trastuzumab-dkst, pertuzumab, margetuximab, PRS343, and ARX788. In embodiments, the antibody capable of binding HER2 is trastuzumab.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other

carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand. In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment. In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

Another aspect of the present invention is method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. In this aspect, the subject has undergone or is undergoing treatment with an antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), or human epidermal growth factor receptor 2 (Her2), or and capable of, respectively, inhibiting the interaction of CD20, EGFR, or Her2, with one or more of its ligands.

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with an antibody that is capable of binding CD20, EGFR, or Her2.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody capable of binding CD20 is selected from rituximab, obinutuzumab, ofatumumab, ocrelizumab, ocaratuzumab, and veltuzumab. In embodiments, the antibody capable of binding CD20 is rituximab.

In embodiments, the antibody capable of binding EGFR is selected from cetuximab, ABP 494 (Actavis), CT-P15 (Celltrion), STI-001 (Sorrento), panitumumab, necitumumab, nimotuzumab, matuzumab, and chimeric 806 (ch806). In embodiments, the antibody capable of binding EGFR is cetuximab.

In embodiments, the antibody capable of binding HER2 is selected from trastuzumab, trastuzumab deruxtecan, ado-trastuzumab emtansine (T-DM1), trastuzumab-pkrb, trastuzumab-dkst, pertuzumab, margetuximab, PRS343, and ARX788. In embodiments, the antibody capable of binding HER2 is trastuzumab.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous

carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand. In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment. In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

Yet another aspect of the present invention is a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), or human epidermal growth factor receptor 2 (Her2), or and capable of, respectively, inhibiting the interaction of CD20, EGFR, or Her2, with one or more of its ligands. In this aspect, the subject has undergone or is undergoing treatment with: a heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with the heterologous chimeric protein.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),
(b) a second domain comprising a portion of CD40L, and
(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),
(b) a second domain comprising a portion of OX40L, and
(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),
(b) a second domain comprising a portion of LIGHT, and
(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody capable of binding CD20 is selected from rituximab, obinutuzumab, ofatumumab, ocrelizumab, ocaratuzumab, and veltuzumab. In embodiments, the antibody capable of binding CD20 is rituximab.

In embodiments, the antibody capable of binding EGFR is selected from cetuximab, ABP 494 (Actavis), CT-P15 (Celltrion), STI-001 (Sorrento), panitumumab, necitumumab, nimotuzumab, matuzumab, and chimeric 806 (ch806). In embodiments, the antibody capable of binding EGFR is cetuximab.

In embodiments, the antibody capable of binding HER2 is selected from trastuzumab, trastuzumab deruxtecan, ado-trastuzumab emtansine (T-DM1), trastuzumab-pkrb, trastuzumab-dkst, pertuzumab, margetuximab, PRS343, and ARX788. In embodiments, the antibody capable of binding HER2 is trastuzumab.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand. In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment. In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

In an aspect, the present invention provides a method for treating a cancer in a subject in need thereof. The method comprising steps of providing the subject a first pharmaceutical composition comprising an antibody that is capable of

binding cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and providing the subject a second pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the first pharmaceutical composition and the second pharmaceutical composition are provided simultaneously, the first pharmaceutical composition is provided after the second pharmaceutical composition is provided, or the first pharmaceutical composition is provided before the second pharmaceutical composition is provided.

In embodiments, the dose of the first pharmaceutical composition is less than the dose of the first pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the second pharmaceutical composition.

In embodiments, the dose of the second pharmaceutical composition provided is less than the dose of the second pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the second pharmaceutical composition.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG1 or IgG4, *e.g.,* human IgG1 or human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody that is capable of binding CTLA-4 is selected from the group consisting of YERVOY (ipilimumab), 9D9, tremelimumab (formerly ticilimumab, CP-675,206; MedImmune), AGEN1884, and RG2077.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high

grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

In another aspect, the present invention provides a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. In this aspect, the subject has undergone or is undergoing treatment with an antibody that is capable of binding cytotoxic T lymphocyte-associated antigen 4 (CTLA-4).

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with an antibody that is capable of binding CTLA-4.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG1 or IgG4, *e.g.,* human IgG1 or human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody that is capable of binding CTLA-4 is selected from the group consisting of YERVOY (ipilimumab), 9D9, tremelimumab (formerly ticilimumab, CP-675,206; MedImmune), AGEN1884, and RG2077.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular

cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

In yet another aspect, the present invention provides a method for treating a cancer in a subject comprising: providing the subject a pharmaceutical composition comprising an antibody that is capable of binding cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). In this aspect, the subject has undergone or is undergoing treatment with a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with the heterologous chimeric protein.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG1 or IgG4, *e.g.,* human IgG1 or human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody that is capable of binding CTLA-4 is selected from the group consisting of YERVOY (ipilimumab), 9D9, tremelimumab (formerly ticilimumab, CP-675,206; MedImmune), AGEN1884, and RG2077.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous

carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

An aspect of the present invention is a method for treating a cancer in a subject in need thereof. The method comprising steps of: providing the subject a first pharmaceutical composition comprising a stimulator of interferon genes (STING) agonist, and providing the subject a second pharmaceutical composition comprising a heterologous chimeric protein. In this aspect, the heterologous chimeric protein comprises: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the first pharmaceutical composition and the second pharmaceutical composition are provided simultaneously, the first pharmaceutical composition is provided after the second pharmaceutical composition is provided, or the first pharmaceutical composition is provided before the second pharmaceutical composition is provided.

In embodiments, the dose of the first pharmaceutical composition is less than the dose of the first pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the second pharmaceutical composition.

In embodiments, the dose of the second pharmaceutical composition provided is less than the dose of the second pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the second pharmaceutical composition.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the STING Agonist is selected from the group consisting of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), MIW815(ADU-S100), CRD5500, MK-1454, SB11285, IMSA101, and any STING agonist described in US20140341976, US20180028553, US20180230178, US9549944, WO2015185565, WO2016120305, WO2017044622, WO2017027645, WO2017027646, WO2017093933, WO2017106740, WO2017123657, WO2017123669, WO2017161349, WO2017175147, WO2017175156, WO2017176812, WO2018009466, WO2018045204, WO2018060323, WO2018098203, WO2018100558, WO2018138684, WO2018138685, WO2018152450, WO2018152453, WO2018172206, WO2018198084, WO2018234805, WO2018234807,

WO2018234808, WO2019023459, WO2019046496, WO2019046498, WO2019046500, WO2019074887, WO2019079261, WO2019118839, WO2019125974, or WO2019160884, he contents of which are incorporated herein by reference in their entireties.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand. In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment. In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

Another aspect of the present invention is a method for treating a cancer in a subject. The method comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and (c) a linker linking the first domain

and the second domain. In this aspect, the subject has undergone or is undergoing treatment with a stimulator of interferon genes (STING) agonist.

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with a STING agonist.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the STING Agonist is selected from the group consisting of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), MIW815(ADU-S100), CRD5500, MK-1454, SB11285, IMSA101, and any STING agonist described in US20140341976, US20180028553, US20180230178, US9549944, WO2015185565, WO2016120305, WO2017044622, WO2017027645, WO2017027646, WO2017093933, WO2017106740, WO2017123657, WO2017123669, WO2017161349, WO2017175147, WO2017175156, WO2017176812, WO2018009466, WO2018045204, WO2018060323, WO2018098203, WO2018100558, WO2018138684, WO2018138685, WO2018152450, WO2018152453, WO2018172206, WO2018198084, WO2018234805, WO2018234807, WO2018234808, WO2019023459, WO2019046496, WO2019046498, WO2019046500, WO2019074887, WO2019079261, WO2019118839, WO2019125974, or WO2019160884, he contents of which are incorporated herein by reference in their entireties.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma;

hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand. In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment. In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

Yet another aspect of the present invention is a method for treating a cancer in a subject. The method comprising providing the subject a pharmaceutical composition comprising a stimulator of interferon genes (STING) agonist. In this aspect, the subject has undergone or is undergoing treatment with a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with the heterologous chimeric protein.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the STING Agonist is selected from the group consisting of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), MIW815(ADU-S100), CRD5500, MK-1454, SB11285, IMSA101, and any STING agonist described in US20140341976, US20180028553, US20180230178, US9549944, WO2015185565, WO2016120305, WO2017044622, WO2017027645, WO2017027646, WO2017093933, WO2017106740, WO2017123657, WO2017123669, WO2017161349, WO2017175147, WO2017175156, WO2017176812, WO2018009466, WO2018045204, WO2018060323, WO2018098203, WO2018100558, WO2018138684, WO2018138685, WO2018152450, WO2018152453, WO2018172206, WO2018198084, WO2018234805, WO2018234807, WO2018234808, WO2019023459, WO2019046496, WO2019046498, WO2019046500, WO2019074887, WO2019079261, WO2019118839, WO2019125974, or WO2019160884, he contents of which are incorporated herein by reference in their entireties.

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular

cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand. In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment. In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

In an aspect, the present invention provides a method for treating a cancer in a subject in need thereof. The method comprising: providing the subject a first pharmaceutical composition comprising a heterologous chimeric protein and providing the subject a second pharmaceutical composition comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand and capable of inhibiting the interaction of PD-1 with one or more of its ligands. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the first pharmaceutical composition and the second pharmaceutical composition are provided simultaneously, the first pharmaceutical composition is provided after the second pharmaceutical composition is provided, or the first pharmaceutical composition is provided before the second pharmaceutical composition is provided.

In embodiments, the dose of the first pharmaceutical composition is less than the dose of the first pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the second pharmaceutical composition.

In embodiments, the dose of the second pharmaceutical composition provided is less than the dose of the second pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the first pharmaceutical composition.

In embodiments, the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the second pharmaceutical composition.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO 4538, BMS 936558, MDX1106, OPDIVO (Bristol Myers Squibb)), pembrolizumab (KEYTRUDA/MK 3475, Merck), and cemiplimab ((REGN-2810).

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS

936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

In another aspect, the present invention provides a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain. In this aspect, the subject has undergone or is undergoing treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand and capable of inhibiting the interaction of PD-1 with one or more of its ligands.

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO 4538, BMS 936558, MDX1106, OPDIVO (Bristol Myers Squibb)), pembrolizumab (KEYTRUDA/MK 3475, Merck), and cemiplimab ((REGN-2810).

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

In yet another aspect, the present invention provides a method for treating a cancer in a subject comprising providing the subject a pharmaceutical composition comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand and capable of inhibiting the interaction of PD-1 with one or more of its ligands. In this aspect, the subject has undergone or is undergoing treatment with a heterologous chimeric protein. The heterologous chimeric protein comprising: (a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand, (b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and (c) a linker linking the first domain and the second domain.

In embodiments, the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with the heterologous chimeric protein.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of OX40L.

In embodiments, the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of LIGHT.

In embodiments, the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

In embodiments, the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain. In embodiments, the linker comprises a hinge-CH2-CH3 Fc domain derived

from IgG4, *e.g.,* human IgG4. In embodiments, the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of OX40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO 4538, BMS 936558, MDX1106, OPDIVO (Bristol Myers Squibb)), pembrolizumab (KEYTRUDA/MK 3475, Merck), and cemiplimab ((REGN-2810).

In embodiments, the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other

carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

In embodiments, the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

In embodiments, the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

Combination Therapies and Conjugation

In embodiments, the invention provides for chimeric proteins and methods that further comprise administering an additional agent to a subject. In embodiments, the invention pertains to co-administration and/or co-formulation. Any of the compositions disclosed herein may be co-formulated and/or co-administered.

In embodiments, any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention disclosed herein acts synergistically when co-administered with another agent and is administered at doses that are lower than the doses commonly employed when such agents are used as monotherapy. In embodiments, any agent referenced herein may be used in combination with any of the chimeric proteins disclosed herein.

In aspects and embodiments of the present invention, the patient in need of a cancer treatment comprising an antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention, as disclosed herein, has been treated with, is contemporaneously treated with, or is subsequently treated with another anti-cancer therapy, as disclosed herein.

The other anti-cancer therapy may comprise radiotherapy.

The other anti-cancer therapy may include a synthetic polypeptide comprising at least one domain capable of binding an immune checkpoint molecule. In embodiments, the immune checkpoint molecule is selected from PD-1, PD-L1, PD-L2, ICOS, ICOSL, and CTLA-4.

The other anti-cancer therapy may include a synthetic polypeptide comprising at least one domain capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20.

The other anti-cancer therapy may be surgery to excise the cancer, *i.e.,* tumor.

The other anti-cancer therapy may include a cell-based immuno-oncology therapy, *e.g.,* chimeric antigen receptor T cell (CAR-T).

The other anti-cancer therapy may include administration of one more chemotherapeutic agents.

In aspects and embodiments of the present invention, the one or more chemotherapeutic agent selected from 5-FU (Fluorouracil), Abemaciclib, Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, Acalabrutinib, AC-T, ADE, Adriamycin (Doxorubicin), Afatinib Dimaleate, Afinitor (Everolimus), Afinitor Difsperz (Everolimus), Akynzeo (Netupitant and Palonosetron), Aldara (Imiquimod), Aldesleukin, Alecensa (Alectinib), Alectinib, Alimta (PEMETREXED), Aliqopa (Copanlisib Hydrochloride), Alkeran (Melphalan), Aloxi (Palonosetron Hydrochloride), Alunbrig (Brigatinib), Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Amifostine, Aminolevulinic Acid, Anastrozole, Aprepitant, Aredia (Pamidronate), Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Asparaginase Erwinia chrysanthemi, Axicabtagene Ciloleucel, Axitinib, Azacitidine, BEACOPP, Becenum (Carmustine), Beleodaq (Belinostat), Belinostat, Bendamustine Hydrochloride, BEP, Bexarotene, Bicalutamide, BiCNU (Carmustine), Blenoxane (Bleomycin), Bortezomib, Bosulif (Bosutinib), Bosutinib, Brigatinib, BuMel, Busulfan, Busulfex (Busulfan)C, Cabazitaxel, Cabometyx (Cabozantinib), Cabozantinib-S-Malate, CAF, Calquence (Acalabrutinib), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Caprelsa (Vandetanib), Carac (Fluorouracil--Topical), Carboplatin, CARBOPLATIN-TAXOL, Carfilzomib, Carmubris (Carmustine), Carmustine, Casodex (Bicalutamide), CeeNU (Lomustine), CEM, Ceritinib, Cerubidine (Daunorubicin), Cervarix (Recombinant HPV Bivalent Vaccine), CEV, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Cladribine, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cobimetinib, Cometriq (Cabozantinib), Copanlisib Hydrochloride, COPDAC, COPP, COPP-ABV, Cosmegen (Dactinomycin), Cotellic (Cobimetinib), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cytarabine, Cytarabine Liposome, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Cytoxan (Cytoxan), Dabrafenib, Dacarbazine, Dacogen (Decitabine), Dactinomycin, Dasatinib, Daunorubicin Hydrochloride, Daunorubicin Hydrochloride and Cytarabine Liposome, DaunoXome (Daunorubicin Lipid Complex), Decadron (Dexamethasone), Decitabine, Defibrotide Sodium, Defitelio (Defibrotide Sodium), Degarelix, Denileukin Diftitox, DepoCyt (Cytarabine Liposome), Dexamethasone, Dexamethasone Intensol (Dexamethasone), Dexpak Taperpak (Dexamethasone), Dexrazoxane Hydrochloride, Docefrez (Docetaxel), Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), Droxia (Hydroxyurea), DTIC (Decarbazine), DTIC-Dome (Dacarbazine), Efudex (Fluorouracil--Topical), Eligard (Leuprolide), Elitek (Rasburicase), Ellence (Ellence (epirubicin)), Eloxatin (Oxaliplatin), Elspar (Asparaginase), Eltrombopag Olamine, Emcyt (Estramustine), Emend (Aprepitant), Enasidenib Mesylate, Enzalutamide, Epirubicin Hydrochloride, EPOCH, Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase

Erwinia chrysanthemi), Ethyol (Amifostine), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Eulexin (Flutamide), Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Evomela (Melphalan Hydrochloride), Exemestane, Fareston (Toremifene), Farydak (Panobinostat), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Firmagon (Degarelix), FloPred (Prednisolone), Fludara (Fludarabine), Fludarabine Phosphate, Fluoroplex (Fluorouracil), Fluorouracil, Flutamide, Folex (Methotrexate), Folex PFS (Methotrexate), FOLFIRI, FOLFIRINOX, FOLFOX, Folotyn (Pralatrexate), FUDR (FUDR (floxuridine)), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gardasil 9 (Recombinant HPV Nonavalent Vaccine), Gefitinib, Gemcitabine Hydrochloride, GEMCITABINE-CISPLATIN, GEMCITABINE-OXALIPLATIN, Gemzar (Gemcitabine), Gilotrif (Afatinib Dimaleate), Gilotrif (Afatinib), Gleevec (Imatinib Mesylate), Gliadel (Carmustine), Glucarpidase, Goserelin Acetate, Halaven (Eribulin Mesylate), Hemangeol (Propranolol Hydrochloride), Hexalen (Altretamine), HPV Bivalent Vaccine, Recombinant, HPV Nonavalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Hycamtin (Topotecan), Hydrea (Hydroxyurea), Hydroxyurea, Hyper-CVAD, Ibrance (Palbociclib), Ibrutinib, ICE, Iclusig (Ponatinib), Idamycin PFS (Idarubicin), Idarubicin Hydrochloride, Idelalisib, Idhifa (Enasidenib), Ifex (Ifosfamide), Ifosfamide, Ifosfamidum (Ifosfamide), Imatinib Mesylate, Imbruvica (Ibrutinib), Imiquimod, Imlygic (Talimogene Laherparepvec), Inlyta (Axitinib), Iressa (Gefitinib), Irinotecan Hydrochloride, Irinotecan Hydrochloride Liposome, Istodax (Romidepsin), Ixabepilone, Ixazomib Citrate, Ixempra (Ixabepilone), Jakafi (Ruxolitinib Phosphate), Jakafi (Ruxolitinib), JEB, Jevtana (Cabazitaxel), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Kisqali (Ribociclib), Kyprolis (Carfilzomib), Lanreotide Acetate, Lanvima (Lenvatinib), Lapatinib Ditosylate, Lenalidomide, Lenvatinib Mesylate, Lenvima (Lenvatinib Mesylate), Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leukine (Sargramostim), Leuprolide Acetate, Leustatin (Cladribine), Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Lomustine, Lonsurf (Trifluridine and Tipiracil), Lupron (Leuprolide), Lynparza (Olaparib), Lysodren (Mitotane), Marqibo (Vincristine Sulfate Liposome), Marqibo Kit (Vincristine Lipid Complex), Matulane (Procarbazine), Mechlorethamine Hydrochloride, Megace (Megestrol), Megestrol Acetate, Mekinist (Trametinib), Melphalan, Melphalan Hydrochloride, Mercaptopurine, Mesnex (Mesna), Metastron (Strontium-89 Chloride), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Methylnaltrexone Bromide, Mexate (Methotrexate), Mexate-AQ (Methotrexate), Midostaurin, Mitomycin C, Mitoxantrone Hydrochloride, Mitozytrex (Mitomycin C), MOPP, Mostarina (Prednimustine), Mozobil (Plerixafor), Mustargen (Mechlorethamine), Mutamycin (Mitomycin), Myleran (Busulfan), Mylosar (Azacitidine), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Navelbine (Vinorelbine), Nelarabine, Neosar (Cyclophosphamide), Neratinib Maleate, Nerlynx (Neratinib), Netupitant and Palonosetron Hydrochloride, Neulasta (filgrastim), Neulasta (pegfilgrastim), Neupogen (filgrastim), Nexavar (Sorafenib), Nilandron (Nilutamide), Nilotinib, Nilutamide, Ninlaro (Ixazomib), Nipent (Pentostatin), Niraparib Tosylate Monohydrate, Nolvadex (Tamoxifen), Novantrone (Mitoxantrone), Nplate (Romiplostim), Odomzo (Sonidegib), OEPA, OFF, Olaparib, Omacetaxine Mepesuccinate, Oncaspar (Pegaspargase), Oncovin (Vincristine), Ondansetron

Hydrochloride, Onivyde (Irinotecan Hydrochloride Liposome), Ontak (Denileukin Diftitox), Onxol (Paclitaxel), OPPA, Orapred (Prednisolone), Osimertinib, Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, PAD, Palbociclib, Palifermin, Palonosetron Hydrochloride, Palonosetron Hydrochloride and Netupitant, Pamidronate Disodium, Panobinostat, Panretin (Alitretinoin), Paraplat (Carboplatin), Pazopanib Hydrochloride, PCV, PEB, Pediapred (Prednisolone), Pegaspargase, Pegfilgrastim, Pemetrexed Disodium, Platinol (Cisplatin), PlatinolAQ (Cisplatin), Plerixafor, Pomalyst (Pomalidomide), Ponatinib Hydrochloride, Pralatrexate, Prednisone, Procarbazine Hydrochloride, Proleukin (Aldesleukin), Promacta (Eltrombopag Olamine), Propranolol Hydrochloride, Purinethol (Mercaptopurine), Purixan (Mercaptopurine), Radium 223 Dichloride, Raloxifene Hydrochloride, Rasburicase, R-CHOP, R-CVP, Reclast (Zoledronic acid), Recombinant Human Papillomavirus (HPV) Bivalent Vaccine, Recombinant Human Papillomavirus (HPV) Nonavalent Vaccine, Recombinant Human Papillomavirus (HPV) Quadrivalent Vaccine, Regorafenib, Relistor (Methylnaltrexone Bromide), R-EPOCH, Revlimid (Lenalidomide), Rheumatrex (Methotrexate), Ribociclib, R-ICE, Rolapitant Hydrochloride, Romidepsin, Romiplostim, Rubex (Doxorubicin), Rubidomycin (Daunorubicin Hydrochloride), Rubraca (Rucaparib), Rucaparib Camsylate, Ruxolitinib Phosphate, Rydapt (Midostaurin), Sandostatin (Octreotide), Sandostatin LAR Depot (Octreotide), Sclerosol Intrapleural Aerosol (Talc), Soltamox (Tamoxifen), Somatuline Depot (Lanreotide Acetate), Sonidegib, Sorafenib Tosylate, Sprycel (Dasatinib), STANFORD V, Sterapred (Prednisone), Sterapred DS (Prednisone), Sterile Talc Powder (Talc), Steritalc (Talc), Sterecyst (Prednimustine), Stivarga (Regorafenib), Sunitinib Malate, Supprelin LA (Histrelin), Sutent (Sunitinib Malate), Sutent (Sunitinib), Synribo (Omacetaxine Mepesuccinate), Tabloid (Thioguanine), TAC, Tafinlar (Dabrafenib), Tagrisso (Osimertinib), Talc, Talimogene Laherparepvec, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib), Targretin (Bexarotene), Tasigna (Decarbazine), Tasigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Tepadina (Thiotepa), Thalidomide, Thalomid (Thalidomide), TheraCys BCG (BCG), Thioguanine, Thioplex (Thiotepa), Thiotepa, TICE BCG (BCG), Tisagenlecleucel, Tolak (Fluorouracil--Topical), Toposar (Etoposide), Topotecan Hydrochloride, Toremifene, Torisel (Temsirolimus), Totect (Dexrazoxane Hydrochloride), TPF, Trabectedin, Trametinib, Treanda (Bendamustine hydrochloride), Trelstar (Triptorelin), Trexall (Methotrexate), Trifluridine and Tipiracil Hydrochloride, Trisenox (Arsenic trioxide), Tykerb (lapatinib), Uridine Triacetate, VAC, Valrubicin, Valstar (Valrubicin Intravesical), Valstar (Valrubicin), VAMP, Vandetanib, Vantas (Histrelin), Varubi (Rolapitant), VeIP, Velban (Vinblastine), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, Venclexta (Venetoclax), Vepesid (Etoposide), Verzenio (Abemaciclib), Vesanoid (Tretinoin), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine), Vincrex (Vincristine), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, VIP, Vismodegib, Vistogard (Uridine Triacetate), Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib), Vumon (Teniposide), Vyxeos (Daunorubicin Hydrochloride and Cytarabine Liposome), W, Wellcovorin (Leucovorin Calcium), Wellcovorin IV (Leucovorin), Xalkori (Crizotinib), XELIRI, Xeloda (Capecitabine), XELOX, Xofigo (Radium 223 Dichloride), Xtandi (Enzalutamide), Yescarta (Axicabtagene Ciloleucel), Yondelis (Trabectedin), Zaltrap (Ziv-Aflibercept), Zanosar (Streptozocin), Zarxio

(Filgrastim), Zejula (Niraparib), Zelboraf (Vemurafenib), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zofran (Ondansetron Hydrochloride), Zoladex (Goserelin), Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic acid), Zortress (Everolimus), Zydelig (Idelalisib), Zykadia (Ceritinib), Zytiga (Abiraterone Acetate), and Zytiga (Abiraterone).

In embodiments, any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention disclosed herein may be used in combination with any of the anti-cancer therapy disclosed herein.

In embodiments, any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention disclosed herein acts synergistically when co-administered with another anti-cancer therapy (*e.g.,* radiotherapy and/or a chemotherapeutic agent); resulting in, for example, the other anti-cancer therapy is administered at doses that are lower than the doses commonly employed when the other anti-cancer therapy is are used as monotherapy. In embodiments, the chimeric protein, as disclosed herein, reduces the number of administrations of the co-administered anti-cancer therapy.

In aspects and embodiments of the present invention, a patient in need of a cancer treatment comprising an antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention, as disclosed herein, is or is predicted to be poorly responsive or is non-responsive to an immunotherapy, *e.g.,* an anti-cancer immunotherapy, as disclosed herein. Moreover, in embodiments, a patient in need of an anti-cancer agent, as disclosed herein, is or may is predicted to be poorly responsive or non-responsive to an immune checkpoint immunotherapy. The immune checkpoint molecule may be selected from PD-1, PD-L1, PD-L2, ICOS, ICOSL, and CTLA-4. Moreover, in embodiments, a patient in need of an anti-cancer agent, as disclosed herein, is or may is predicted to be poorly responsive or non-responsive to an therapy directed to one or more of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), and CD20.

In embodiments, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) disclosed herein, include derivatives that are modified, *i.e.,* by the covalent attachment of any type of molecule to the composition such that covalent attachment does not prevent the activity of the composition. For example, but not by way of limitation, derivatives include composition that have been modified by, *inter alia*, glycosylation, lipidation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, *etc.* Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.* Additionally, the derivative can contain one or more non-classical amino acids. In still other embodiments, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention

(and/or additional agents) disclosed herein further comprise a cytotoxic agent, comprising, in illustrative embodiments, a toxin, a chemotherapeutic agent, a radioisotope, and an agent that causes apoptosis or cell death. Such agents may be conjugated to a composition disclosed herein.

The antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or other anti-cancer therapy) disclosed herein may thus be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials.

In aspects and embodiments of the present invention, the patient in need of treatment for an inflammatory disease or disorder, has been treated with, is contemporaneously treated with, or is subsequently treated with another agent for treating an inflammatory disease or disorder. Examples of such other agents include a steroidal anti-inflammatory agent, a non-steroidal anti-inflammatory agent (NSAID), and/or an immunosuppressive drug.

Examples of a NSAID include salicylic acid, acetyl salicylic acid, methyl salicylate, glycol salicylate, salicylmides, benzyl-2,5-diacetoxybenzoic acid, ibuprofen, fulindac, naproxen, ketoprofen, etofenamate, phenylbutazone, and indomethacin.

Examples of a steroidal anti-inflammatory agents includes corticosteroids selected from hydroxyltriamcinolone, alpha-methyl dexamethasone, beta-methyl betamethasone, beclomethasone dipropionate, betamethasone benzoate, betamethasone dipropionate, betamethasone valerate, clobetasol valerate, desonide, desoxymethasone, dexamethasone, diflorasone diacetate, diflucortolone valerate, fluadrenolone, fluclorolone acetonide, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine butylester, fluocortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters, chloroprednisone, clocortelone, clescinolone, dichlorisone, difluprednate, flucloronide, flunisolide, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate.

A steroidal anti-inflammatory agent may likewise have activity as an immunosuppressive drug.

Other examples of immunosuppressive drug include cytostatics such as alkylating agents, antimetabolites (*e.g.,* azathioprine, methotrexate), cytotoxic antibiotics, antibodies (*e.g.,* basiliximab, daclizumab, and muromonab), anti-immunophilins (*e.g.,* cyclosporine, tacrolimus, sirolimus), inteferons, opioids, TNF binding proteins, mycophenolates, and small biological agents (*e.g.,* fingolimod, myriocin).

In embodiments, a patient in need of an agent for treating an autoimmune disease or disorder, has been treated with, is contemporaneously treated with, or is subsequently treated with a steroidal anti-inflammatory agent, a non-steroidal anti-inflammatory agent, and/or an immunosuppressive drug, as disclosed elsewhere herein.

In embodiments, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or other agent for treating an inflammatory disease or disorder) disclosed herein, include derivatives that are modified, *i.e.,* by the covalent attachment of any type of molecule to the composition such that covalent attachment does not prevent the activity of the composition. For example, but not by way of limitation, derivatives include composition that have been modified by, inter alia, glycosylation, lipidation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of turicamycin, etc. Additionally, the derivative can contain one or more non-classical amino acids.

The antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or other agent for treating an inflammatory disease or disorder) disclosed herein may thus be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials.

Pharmaceutical composition

The methods of the present invention include administering pharmaceutical compositions comprising a therapeutically effective amount of, at least one, antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention, as disclosed herein.

The antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) disclosed herein can possess a sufficiently basic functional group, which can react with an inorganic or organic acid, or a carboxyl group, which can react with an inorganic or organic base, to form a pharmaceutically acceptable salt. A pharmaceutically-acceptable acid addition salt is formed from a pharmaceutically acceptable acid, as is well known in the art. Such salts include the pharmaceutically acceptable salts listed in, for example, *Journal of Pharmaceutical Science*, 66, 2-19 (1977) and *The Handbook of Pharmaceutical Salts; Properties, Selection, and Use*. P. H. Stahl and C. G. Wermuth (eds.), Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety.

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

Further, any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can be administered to a subject as a component of a composition, *e.g.,* pharmaceutical composition, that comprises a pharmaceutically acceptable carrier or vehicle. Such pharmaceutical compositions can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration. Pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical excipients can be, for example, saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used. In embodiments, the pharmaceutically acceptable excipients are sterile when administered to a subject. Water is a useful excipient when any agent disclosed herein is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, specifically for injectable solutions. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Any agent disclosed herein, if desired, can also comprise minor amounts of wetting or emulsifying agents, or pH buffering agents.

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

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

The present invention includes the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) in various formulations of pharmaceutical composition. Any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can take the form of solutions, suspensions, emulsion, drops, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any

other form suitable for use. DNA or RNA constructs encoding the protein sequences may also be used. In embodiments, the composition is in the form of a capsule (*see, e.g.,* U.S. Patent No. 5,698,155). Other examples of suitable pharmaceutical excipients are described in *Remington's Pharmaceutical Sciences* 1447-1676 (Alfonso R. Gennaro eds., 19th ed. 1995), incorporated herein by reference.

Where necessary, the pharmaceutical compositions comprising the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) can also include a solubilizing agent. Also, the agents can be delivered with a suitable vehicle or delivery device as known in the art. Combination therapies outlined herein can be co-delivered in a single delivery vehicle or delivery device. Compositions for administration can optionally include a local anesthetic such as, for example, lignocaine to lessen pain at the site of the injection.

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

In embodiments, any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein is formulated in accordance with routine procedures as a pharmaceutical composition adapted for a mode of administration disclosed herein.

Administration, Dosing, and Treatment Regimens

Routes of administration include, for example: intradermal, intratumoral, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin.

As examples, administration results in the release of antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) disclosed herein into the bloodstream (*via* enteral or parenteral administration), or alternatively, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) is administered directly to the site of active disease.

Any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can be administered orally. Such antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) can also be administered by any other convenient route, for example, by intravenous infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.,* oral mucosa, rectal and intestinal mucosa, *etc.*) and can be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, *e.g.,* encapsulation in liposomes, microparticles, microcapsules, capsules, *etc.*, and can be used to administer.

In specific embodiments, it may be desirable to administer locally to the area in need of treatment. In embodiments, for instance in the treatment of cancer, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) are administered in the tumor microenvironment (*e.g.,* cells, molecules, extracellular matrix and/or blood vessels that surround and/or feed a tumor cell, inclusive of, for example, tumor vasculature; tumor-infiltrating lymphocytes; fibroblast reticular cells; endothelial progenitor cells (EPC); cancer-associated fibroblasts; pericytes; other stromal cells; components of the extracellular matrix (ECM); dendritic cells; antigen presenting cells; T-cells; regulatory T cells; macrophages; neutrophils; and other immune cells located proximal to a tumor) or lymph node and/or targeted to the tumor microenvironment or lymph node. In embodiments, for instance in the treatment of cancer, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention (and/or additional agents) are administered intratumorally.

In embodiments, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention allows for a dual effect that provides less side effects than are seen in conventional immunotherapy (e.g., treatments with one or more of OPDIVO, KEYTRUDA, YERVOY, and TECENTRIQ). For example, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention reduce or prevent commonly observed immune-related adverse events that affect various tissues and organs including the skin, the gastrointestinal tract, the kidneys, peripheral and central nervous system, liver, lymph nodes, eyes, pancreas, and the endocrine system; such as hypophysitis, colitis, hepatitis, pneumonitis, rash, and rheumatic disease. Further, the present local administration, e.g., intratumorally, obviate adverse event seen with standard systemic administration, e.g., IV infusions, as are used with conventional immunotherapy (e.g., treatments with one or more of OPDIVO, KEYTRUDA, YERVOY, and TECENTRIQ).

Dosage forms suitable for parenteral administration (*e.g.,* intravenous, intramuscular, intraperitoneal, subcutaneous and intra-articular injection and infusion) include, for example, solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions (*e.g.,* lyophilized composition), which can

be dissolved or suspended in sterile injectable medium immediately before use. They may contain, for example, suspending or dispersing agents known in the art.

The dosage of any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein as well as the dosing schedule can depend on various parameters, including, but not limited to, the disease being treated, the subject's general health, and the administering physician's discretion. Any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention, disclosed herein, can be administered prior to (*e.g.,* 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concurrently with, or subsequent to (*e.g.,* 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of an additional agent, to a subject in need thereof.

In embodiments, an antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention and an additional agent(s) are administered 1 minute apart, 10 minutes apart, 30 minutes apart, less than 1 hour apart, 1 hour apart, 1 hour to 2 hours apart, 2 hours to 3 hours apart, 3 hours to 4 hours apart, 4 hours to 5 hours apart, 5 hours to 6 hours apart, 6 hours to 7 hours apart, 7 hours to 8 hours apart, 8 hours to 9 hours apart, 9 hours to 10 hours apart, 10 hours to 11 hours apart, 11 hours to 12 hours apart, 1 day apart, 2 days apart, 3 days apart, 4 days apart, 5 days apart, 6 days apart, 1 week apart, 2 weeks apart, 3 weeks apart, or 4 weeks apart.

In embodiments, the present invention relates to the co-administration of an antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention which induces an innate immune response and another antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention which induces an adaptive immune response. In such embodiments, the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention which induces an innate immune response may be administered before, concurrently with, or subsequent to administration of the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention which induces an adaptive immune response. For example, the antibodies directed to immune checkpoint molecules; STING agonists; and/or chimeric proteins used in methods of the present invention may be administered 1 minute apart, 10 minutes apart, 30 minutes apart, less than 1 hour apart, 1 hour apart, 1 hour to 2 hours apart, 2 hours to 3 hours apart, 3 hours to 4 hours apart, 4 hours to 5 hours apart, 5 hours

to 6 hours apart, 6 hours to 7 hours apart, 7 hours to 8 hours apart, 8 hours to 9 hours apart, 9 hours to 10 hours apart, 10 hours to 11 hours apart, 11 hours to 12 hours apart, 1 day apart, 2 days apart, 3 days apart, 4 days apart, 5 days apart, 6 days apart, 1 week apart, 2 weeks apart, 3 weeks apart, or 4 weeks apart. In an illustrative embodiment, the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention which induces an innate immune response and the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention which induces an adaptive response are administered 1 week apart, or administered on alternate weeks (*i.e.,* administration of the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention inducing an innate immune response is followed 1 week later with administration of the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention which induces an adaptive immune response and so forth).

The dosage of any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can depend on several factors including the severity of the condition, whether the condition is to be treated or prevented, and the age, weight, and health of the subject to be treated. Additionally, pharmacogenomic (the effect of genotype on the pharmacokinetic, pharmacodynamic or efficacy profile of a therapeutic) information about a particular subject may affect dosage used. Furthermore, the exact individual dosages can be adjusted somewhat depending on a variety of factors, including the specific combination of the agents being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the particular disease being treated, the severity of the disorder, and the anatomical location of the disorder. Some variations in the dosage can be expected.

For administration of any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein by parenteral injection, the dosage may be about 0.1 mg to about 250 mg per day, about 1 mg to about 20 mg per day, or about 3 mg to about 5 mg per day. Generally, when orally or parenterally administered, the dosage of any agent disclosed herein may be about 0.1 mg to about 1500 mg per day, or about 0.5 mg to about 10 mg per day, or about 0.5 mg to about 5 mg per day, or about 200 to about 1,200 mg per day (*e.g.,* about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1,000 mg, about 1,100 mg, about 1,200 mg per day).

In embodiments, administration of the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein is by parenteral injection at a dosage of about 0.1 mg to about 1500 mg per treatment, or about 0.5 mg to about 10 mg per treatment,

or about 0.5 mg to about 5 mg per treatment, or about 200 to about 1,200 mg per treatment (*e.g.,* about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1,000 mg, about 1,100 mg, about 1,200 mg per treatment).

In embodiments, a suitable dosage of the antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) is in a range of about 0.01 mg/kg to about 100 mg/kg of body weight or about 0.01 mg/kg to about 10 mg/kg of body weight of the subject, for example, about 0.01 mg/kg, about 0.02 mg/kg, about 0.03 mg/kg, about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, 1.9 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg body weight, inclusive of all values and ranges therebetween.

In another embodiment, delivery can be in a vesicle, in particular a liposome (*see* Langer, 1990, *Science* 249:1527-1533; Treat *et al.*, in *Liposomes in Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989).

An antibody directed to immune checkpoint molecules; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can be administered by controlled-release or sustained-release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,556, each of which is incorporated herein by reference in its entirety. Such dosage forms can be useful for providing controlled- or sustained-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Controlled- or sustained-release of an active ingredient can be stimulated by various conditions, including but not limited to, changes in pH, changes in temperature, stimulation by an appropriate wavelength of light, concentration or availability of enzymes, concentration or availability of water, or other physiological conditions or compounds.

In another embodiment, polymeric materials can be used (*see Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; *see also* Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, *Ann. Neurol.* 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 71:105).

In another embodiment, a controlled-release system can be placed in proximity of the target area to be treated, thus requiring only a fraction of the systemic dose (*see, e.g.,* Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, *Science* 249:1527-1533) may be used.

Administration of any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can, independently, be one to four times daily or one to four times per month or one to six times per year or once every two, three, four or five years. Administration can be for the duration of one day or one month, two months, three months, six months, one year, two years, three years, and may even be for the life of the subject.

The dosage regimen utilizing any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can be selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the subject; the severity of the condition to be treated; the route of administration; the renal or hepatic function of the subject; the pharmacogenomic makeup of the individual; and the specific compound of the invention employed. Any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention (and/or additional agents) disclosed herein can be administered continuously rather than intermittently throughout the dosage regimen.

Fusion Proteins, Nucleic Acids, and Cells

A chimeric protein used in a method of the present invention may be a recombinant fusion protein, *e.g.,* a single polypeptide having the extracellular domains disclosed herein. For example, in embodiments, the chimeric protein is translated as a single unit in a prokaryotic cell, a eukaryotic cell, or a cell-free expression system.

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

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

Constructs could be produced by cloning of the nucleic acids encoding the three fragments (the extracellular domain of a Type I transmembrane protein, followed by a linker sequence, followed by the extracellular domain of a Type II transmembrane protein) into a vector (plasmid, viral or other) wherein the amino terminus of the complete sequence corresponded to the 'left' side of the molecule containing the extracellular domain of the Type I transmembrane protein and the carboxy terminus of the complete sequence corresponded to the 'right' side of the molecule containing the extracellular domain of Type II transmembrane protein. In embodiments, of chimeric proteins having one of the other configurations, as described elsewhere herein, a construct would comprise three nucleic acids such that the translated chimeric protein produced would have the desired configuration, *e.g.,* a dual inward-facing chimeric protein. Accordingly, in embodiments, the chimeric proteins used in methods of the present invention are engineered as such.

A chimeric protein used in a method of the present invention may be encoded by a nucleic acid cloned into an expression vector. In embodiments, the expression vector comprises DNA or RNA. In embodiments, the expression vector is a mammalian expression vector.

Both prokaryotic and eukaryotic vectors can be used for expression of the chimeric protein. Prokaryotic vectors include constructs based on *E. coli* sequences (*see, e.g.,* Makrides, *Microbiol Rev* 1996, 60:512-538). Non-limiting examples of regulatory regions that can be used for expression in *E. coli* include lac, trp, lpp, phoA, recA, tac, T3, T7 and λP_L. Non-limiting examples of prokaryotic expression vectors may include the λgt vector series such as λgt11 (Huynh *et al.*, in "DNA Cloning Techniques, Vol. I: A Practical Approach," 1984, (D. Glover, ed.), pp. 49-78, IRL Press, Oxford), and the pET vector series (Studier *et al.*, *Methods Enzymol* 1990, 185:60-89). Prokaryotic host-vector systems cannot perform much of the post-translational processing of mammalian cells, however. Thus, eukaryotic host- vector systems may be particularly useful. A variety of regulatory regions can be used for expression of the chimeric proteins in mammalian host cells. For example, the SV40 early and late promoters, the cytomegalovirus (CMV) immediate early promoter, and the Rous sarcoma virus long terminal repeat (RSV-LTR) promoter can be used. Inducible promoters that may be useful in mammalian cells include, without limitation, promoters associated with the metallothionein II gene, mouse mammary tumor virus glucocorticoid responsive long terminal repeats (MMTV-LTR), the β-interferon gene, and the hsp70 gene (*see*, Williams *et al.*, *Cancer Res* 1989, 49:2735-42; and Taylor *et al.*, *Mol Cell Biol* 1990, 10:165-75). Heat shock promoters or stress promoters also may be advantageous for driving expression of the chimeric proteins in recombinant host cells.

In embodiments, expression vectors comprise a nucleic acid encoding the chimeric proteins, or a complement thereof, operably linked to an expression control region, or complement thereof, that is functional in a mammalian cell. The expression control region is capable of driving expression of the operably linked blocking and/or stimulating agent-

encoding nucleic acid such that the blocking and/or stimulating agent is produced in a human cell transformed with the expression vector.

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

Expression control regions are regulatory polynucleotides (sometimes referred to herein as elements), such as promoters and enhancers, that influence expression of an operably linked nucleic acid. An expression control region of an expression vector of the invention is capable of expressing operably linked encoding nucleic acid in a human cell. In embodiments, the cell is a tumor cell. In another embodiment, the cell is a non-tumor cell. In embodiments, the expression control region confers regulatable expression to an operably linked nucleic acid. A signal (sometimes referred to as a stimulus) can increase or decrease expression of a nucleic acid operably linked to such an expression control region. Such expression control regions that increase expression in response to a signal are often referred to as inducible. Such expression control regions that decrease expression in response to a signal are often referred to as repressible. Typically, the amount of increase or decrease conferred by such elements is proportional to the amount of signal present; the greater the amount of signal, the greater the increase or decrease in expression.

In embodiments, the present invention contemplates the use of inducible promoters capable of effecting high level of expression transiently in response to a cue. For example, when in the proximity of a tumor cell, a cell transformed with an expression vector for the chimeric protein (and/or additional agents) comprising such an expression control sequence is induced to transiently produce a high level of the agent by exposing the transformed cell to an appropriate cue. Illustrative inducible expression control regions include those comprising an inducible promoter that is stimulated with a cue such as a small molecule chemical compound. In other examples, the chimeric protein is expressed by a chimeric antigen receptor containing cell or an in vitro expanded tumor infiltrating lymphocyte, under the control of a promoter which is sensitive to antigen recognition by the cell, and leads to local secretion of the chimeric protein in response to tumor antigen recognition. Particular examples can be found, for example, in U.S. Patent Nos. 5,989,910, 5,935,934, 6,015,709, and 6,004,941, each of which is incorporated herein by reference in its entirety.

Expression control regions and locus control regions include full-length promoter sequences, such as native promoter and enhancer elements, as well as subsequences or polynucleotide variants which retain all or part of full-length or non-variant function. As used herein, the term "functional" and grammatical variants thereof, when used in reference to a nucleic acid sequence, subsequence or fragment, means that the sequence has one or more functions of native nucleic acid sequence (*e.g.,* non-variant or unmodified sequence).

As used herein, “operable linkage” refers to a physical juxtaposition of the components so described as to permit them to function in their intended manner. In the example of an expression control element in operable linkage with a nucleic acid, the relationship is such that the control element modulates expression of the nucleic acid. Typically, an expression

control region that modulates transcription is juxtaposed near the 5' end of the transcribed nucleic acid (*i.e.,* "upstream"). Expression control regions can also be located at the 3' end of the transcribed sequence (*i.e.,* "downstream") or within the transcript (*e.g.,* in an intron). Expression control elements can be located at a distance away from the transcribed sequence (*e.g.,* 100 to 500, 500 to 1000, 2000 to 5000, or more nucleotides from the nucleic acid). A specific example of an expression control element is a promoter, which is usually located 5' of the transcribed sequence. Another example of an expression control element is an enhancer, which can be located 5' or 3' of the transcribed sequence, or within the transcribed sequence.

Expression systems functional in human cells are well known in the art; these include viral systems. Generally, a promoter functional in a human cell is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence into mRNA. A promoter will have a transcription-initiating region, which is usually placed proximal to the 5' end of the coding sequence, and, typically, a TATA box located 25-30 base pairs upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A promoter will also typically contain an upstream promoter element (enhancer element), typically located within 100 to 200 base pairs upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated, and can act in either orientation. Of particular use as promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter, herpes simplex virus promoter, and the CMV promoter.

Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-translational cleavage and polyadenylation. Examples of transcription terminator and polyadenylation signals include those derived from SV40. Introns may also be included in expression constructs.

There is a variety of techniques available for introducing nucleic acids into viable cells. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, polymer-based systems, DEAE-dextran, viral transduction, the calcium phosphate precipitation method, *etc.* For *in vivo* gene transfer, a number of techniques and reagents may also be used, including liposomes; natural polymer-based delivery vehicles, such as chitosan and gelatin; viral vectors are also suitable for *in vivo* transduction. In some situations, it is desirable to provide a targeting agent, such as an antibody or ligand specific for a tumor cell surface membrane protein. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.,* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for

example, by Wu *et al.*, J. Biol. Chem. 262, 4429-4432 (1987); and Wagner *et al.*, Proc. Natl. Acad. Sci. USA 87, 3410-3414 (1990).

Where appropriate, gene delivery agents such as, *e.g.,* integration sequences can also be employed. Numerous integration sequences are known in the art (*see*, *e.g.,* Nunes-Duby *et al.*, Nucleic Acids Res. 26:391-406, 1998; Sadwoski, J. Bacteriol., 165:341-357, 1986; Bestor, Cell, 122(3):322-325, 2005; Plasterk *et al.*, TIG 15:326-332, 1999; Kootstra *et al.*, Ann. Rev. Pharm. Toxicol., 43:413-439, 2003). These include recombinases and transposases. Examples include Cre (Sternberg and Hamilton, J. Mol. Biol., 150:467-486, 1981), lambda (Nash, Nature, 247, 543-545, 1974), Flp (Broach, *et al.*, Cell, 29:227-234, 1982), R (Matsuzaki, *et al.*, J. Bacteriology, 172:610-618, 1990), cpC31 (*see*, *e.g.,* Groth *et al.*, J. Mol. Biol. 335:667-678, 2004), sleeping beauty, transposases of the mariner family (Plasterk *et al.*, supra), and components for integrating viruses such as AAV, retroviruses, and antiviruses having components that provide for virus integration such as the LTR sequences of retroviruses or lentivirus and the ITR sequences of AAV (Kootstra *et al.*, Ann. Rev. Pharm. Toxicol., 43:413-439, 2003). In addition, direct and targeted genetic integration strategies may be used to insert nucleic acid sequences encoding the chimeric fusion proteins including CRISPR/CAS9, zinc finger, TALEN, and meganuclease gene-editing technologies.

In embodiments, the expression vectors for the expression of the chimeric proteins (and/or additional agents) are viral vectors. Many viral vectors useful for gene therapy are known (*see*, *e.g.,* Lundstrom, Trends Biotechnol., 21: 1 17, 122, 2003. Illustrative viral vectors include those selected from Antiviruses (LV), retroviruses (RV), adenoviruses (AV), adeno-associated viruses (AAV), and α viruses, though other viral vectors may also be used. For *in vivo* uses, viral vectors that do not integrate into the host genome are suitable for use, such as α viruses and adenoviruses. Illustrative types of α viruses include Sindbis virus, Venezuelan equine encephalitis (VEE) virus, and Semliki Forest virus (SFV). For *in vitro* uses, viral vectors that integrate into the host genome are suitable, such as retroviruses, AAV, and Antiviruses. In embodiments, the invention provides methods of transducing a human cell *in vivo*, comprising contacting a solid tumor *in vivo* with a viral vector of the invention.

Expression vectors can be introduced into host cells for producing the chimeric proteins used in methods of the present invention. Cells may be cultured *in vitro* or genetically engineered, for example. Useful mammalian host cells include, without limitation, cells derived from humans, monkeys, and rodents (*see*, for example, Kriegler in "Gene Transfer and Expression: A Laboratory Manual," 1990, New York, Freeman & Co.). These include monkey kidney cell lines transformed by SV40 (*e.g.,* COS-7, ATCC CRL 1651); human embryonic kidney lines (*e.g.,* 293, 293-EBNA, or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J Gen Virol* 1977, 36:59); baby hamster kidney cells (*e.g.,* BHK, ATCC CCL 10); Chinese hamster ovary-cells-DHFR (*e.g.,* CHO, Urlaub and Chasin, *Proc Natl Acad Sci USA* 1980, 77:4216); DG44 CHO cells, CHO-K1 cells, mouse sertoli cells (Mather, *Biol Reprod* 1980, 23:243-251); mouse fibroblast cells (*e.g.,* NIH-3T3), monkey kidney cells (*e.g.,* CV1 ATCC CCL 70); African green monkey kidney cells. (*e.g.,* VERO-76, ATCC CRL-1587); human cervical carcinoma cells (*e.g.,* HELA, ATCC CCL 2); canine kidney

cells (*e.g.,* MDCK, ATCC CCL 34); buffalo rat liver cells (*e.g.,* BRL 3A, ATCC CRL 1442); human lung cells (*e.g.,* W138, ATCC CCL 75); human liver cells (*e.g.,* Hep G2, HB 8065); and mouse mammary tumor cells (*e.g.,* MMT 060562, ATCC CCL51). Illustrative cancer cell types for expressing the chimeric proteins disclosed herein include mouse fibroblast cell line, NIH3T3, mouse Lewis lung carcinoma cell line, LLC, mouse mastocytoma cell line, P815, mouse lymphoma cell line, EL4 and its ovalbumin transfectant, E.G7, mouse melanoma cell line, B16F10, mouse fibrosarcoma cell line, MC57, and human small cell lung carcinoma cell lines, SCLC#2 and SCLC#7.

Host cells can be obtained from normal or affected subjects, including healthy humans, cancer patients, and patients with an infectious disease, private laboratory deposits, public culture collections such as the American Type Culture Collection (ATCC), or from commercial suppliers.

Cells that can be used for production of the chimeric proteins used in methods of the present invention *in vitro*, *ex vivo*, and/or *in vivo* include, without limitation, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, chimeric antigen receptor expressing T cells, tumor infiltrating lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells (*e.g.,* as obtained from bone marrow), umbilical cord blood, peripheral blood, and fetal liver. The choice of cell type depends on the type of tumor or infectious disease being treated or prevented, and can be determined by one of skill in the art.

Production and purification of Fc-containing macromolecules (such as monoclonal antibodies) has become a standardized process, with minor modifications between products. For example, many Fc containing macromolecules are produced by human embryonic kidney (HEK) cells (or variants thereof) or Chinese Hamster Ovary (CHO) cells (or variants thereof) or in some cases by bacterial or synthetic methods. Following production, the Fc containing macromolecules that are secreted by HEK or CHO cells are purified through binding to Protein A columns and subsequently 'polished' using various methods. Generally speaking, purified Fc containing macromolecules are stored in liquid form for some period of time, frozen for extended periods of time or in some cases lyophilized. In embodiments, production of the chimeric proteins contemplated herein may have unique characteristics as compared to traditional Fc containing macromolecules. In certain examples, the chimeric proteins may be purified using specific chromatography resins, or using chromatography methods that do not depend upon Protein A capture. In embodiments, the chimeric proteins may be purified in an oligomeric state, or in multiple oligomeric states, and enriched for a specific oligomeric state using specific methods. Without being bound by theory, these methods could include treatment with specific buffers including specified salt concentrations, pH and additive compositions. In other examples, such methods could include treatments that favor one oligomeric state over another. The chimeric proteins obtained herein may be additionally 'polished' using methods that are specified in the art. In embodiments, the chimeric proteins are highly stable and able to tolerate a wide range of pH exposure (between pH 3-12), are able to tolerate a large number of freeze/thaw stresses (greater than 3 freeze/thaw cycles) and are able to tolerate extended incubation at high

temperatures (longer than 2 weeks at 40 degrees C). In embodiments, the chimeric proteins are shown to remain intact, without evidence of degradation, deamidation, etc. under such stress conditions.

Subjects and/or Animals

In embodiments, the subject and/or animal is a mammal, *e.g.,* a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, rabbit, sheep, or non-human primate, such as a monkey, chimpanzee, or baboon. In embodiments, the subject and/or animal is a non-mammal, such, for example, a zebrafish. In embodiments, the subject and/or animal may comprise fluorescently tagged cells (with *e.g.,* GFP). In embodiments, the subject and/or animal is a transgenic animal, which comprises a fluorescent cell.

In embodiments, the subject and/or animal is a human. In embodiments, the human is a pediatric human. In embodiments, the human is an adult human. In embodiments, the human is a geriatric human. In embodiments, the human may be referred to as a patient.

In certain embodiments, the human has an age in a range of from about 0 months to about 6 months old, from about 6 to about 12 months old, from about 6 to about 18 months old, from about 18 to about 36 months old, from about 1 to about 5 years old, from about 5 to about 10 years old, from about 10 to about 15 years old, from about 15 to about 20 years old, from about 20 to about 25 years old, from about 25 to about 30 years old, from about 30 to about 35 years old, from about 35 to about 40 years old, from about 40 to about 45 years old, from about 45 to about 50 years old, from about 50 to about 55 years old, from about 55 to about 60 years old, from about 60 to about 65 years old, from about 65 to about 70 years old, from about 70 to about 75 years old, from about 75 to about 80 years old, from about 80 to about 85 years old, from about 85 to about 90 years old, from about 90 to about 95 years old or from about 95 to about 100 years old.

In embodiments, the subject is a non-human animal, and therefore the invention pertains to veterinary use. In a specific embodiment, the non-human animal is a household pet. In another specific embodiment, the non-human animal is a livestock animal.

In embodiments, the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand. In embodiments, the subject has a cancer that is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

Kits and Medicaments

Aspects of the present invention provide kits that can simplify the administration of the pharmaceutical compositions and/or chimeric proteins disclosed herein.

An illustrative kit of the invention comprises any antibody directed to immune checkpoint molecules; any antibody that is capable of binding epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), or CD20; STING agonist; and/or chimeric protein used in methods of the present invention and/or pharmaceutical composition disclosed herein in unit dosage form. In embodiments, the unit dosage form is a container, such as a pre-filled syringe, which can be sterile, containing any agent disclosed herein and a pharmaceutically acceptable carrier, diluent, excipient, or vehicle. The kit can further comprise a label or printed instructions instructing the use of any agent disclosed herein. The kit may also include a lid speculum, topical anesthetic, and a cleaning agent for the administration location. The kit can also further comprise one or more additional agent disclosed herein. In embodiments, the kit comprises a container containing an effective amount of a composition of the invention and an effective amount of another composition, such those disclosed herein.

Aspects of the present invention include use of a chimeric protein as disclosed herein in the manufacture of a medicament, *e.g.,* a medicament for treatment of cancer and/or treatment of an inflammatory disease.

Any aspect or embodiment disclosed herein can be combined with any other aspect or embodiment as disclosed herein.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Production and characterization of SIRPα-Fc-CD40L

The extracellular domain (ECD) of SIRPα and the ECD of CD40L were fused *via* an antibody Fc domain to generate $SIRP\alpha_{ECD}$-Fc-$CD40L_{ECD}$ chimeric proteins. When human proteins were used, the chimeric protein is referred to as hSIRPα-Fc-CD40L; when murine proteins were used, the chimeric protein is referred to as and mSIRPα-Fc-CD40L. Generically, the chimeric protein is referred to as SIRPα-Fc-CD40L. *In silico* structural modeling predicted that each individual domain of the adjoined construct would fold in accordance with the native molecules, which suggested preservation of both binding functions (**FIG. 3A**, top). Mammalian cells were then transfected with the hSIRPα-Fc-CD40L expressing construct, and the secreted protein was purified from conditioned media by affinity chromatography. The purified protein was then analyzed for the presence of each individual domain by Western blotting using anti-SIRPα, anti-Fc, and anti-CD40L antibodies (**FIG. 3A**, bottom). These blots revealed a glycosylated protein that formed a dimer under non-reducing conditions by SDS-PAGE. The reduced and deglycosylated form of the protein migrated at the predicted monomeric molecular weight of 90.1 kDa. To further characterize the native state of hSIRPα-Fc-CD40L in the absence of detergents, electron microscopy was performed and demonstrated the presence of a hexameric species that has been previously described for TNF ligand fusion proteins (**FIG. 3B**). To determine whether SIRPα-Fc-CD40L retained binding to both CD47 and CD40, functional ELISA assays were developed to quantitatively

demonstrate simultaneous binding of its SIRPα-domain to recombinant hCD47 and its CD40L-domain to recombinant hCD40 (**FIG. 3C** and **FIG. 3D**). Next, binding affinities for SIRPα-Fc-CD40L to recombinant CD47, CD40, or Fc receptors were evaluated by surface plasmon resonance (SPR). These studies indicated that hSIRPα-Fc-CD40L bound with 0.628 nM affinity to recombinant human CD47, 4.74 nM affinity to recombinant human CD40, had undetectable binding to FcγR-Ia, IIb and IIIb, while having preserved 2.33 nM binding affinity to FcRn (**FIG. 3E**). High affinity binding of human SIRPα-Fc-CD40L to recombinant cynomolgus macaque CD40 (at 3.24 nM) and CD47 (at 1.7 nM) was determined. Finally, to confirm that SIRPα-Fc-CD40L interacted with native CD47 and CD40 in a similar manner to recombinant CD47 and CD40, CHOK1-hCD47 and CHOK1-hCD40 reporter cell lines were developed (**FIG. 3F**). Flow cytometry studies using these reporter cell lines confirmed that SIRPα-Fc-CD40L bound to CHOK1-CD47 cells (at 31.85 nM EC50) and CHOK1-CD40 cells (at 22.48 nM EC50), but not to parental CHOK1 cells (**FIG. 3G**). A functional ELISA evaluated whether SIRPα-Fc-CD40L could outcompete a commercially available single-sided SIRPα-Fc control for binding to recombinant CD47 (**FIG. 3H**). Human SIRPα-Fc-CD40L efficiently outcompeted SIRPα-Fc, generating an EC50 of 22 nM, comparable to the 14 nM EC50 produced by a commercial CD47 blocking antibody (**FIG. 3H**).

The murine version of SIRPα-Fc-CD40L (referred to as mSIRPα-Fc-CD40L) was generated to assess activity *in vivo*, and was characterized similarly to the human SIRPα-Fc-CD40L; including Western blot (**FIG. 4A**) and dual ELISA binding to murine CD47 and murine CD40 simultaneously (**FIG. 4B**).

Example 2: SIRPα-Fc-CD40L functional activity – CD40L domain

To examine the functional activity of the CD40L domain of SIRPα-Fc-CD40L, a series of *in vitro* functional assays were developed. First, two different NFκB reporter systems determined the relative signaling activity of SIRPα-Fc-CD40L *via* both the canonical and non-canonical NFκB pathways (**FIG. 5A** and **FIG. 5B**). These data indicated that hSIRPα-Fc-CD40L has similar activity as a single-sided hCD40L fusion protein in both reporter systems. Importantly, hSIRPα-Fc-CD40L was present in a soluble form in both assays, and no Fc receptor or other cross-linking agents were present. On the other hand, a CD40 agonist antibody was unable to stimulate NIK/NFκB activity in the absence of an accessory cell that provides Fc receptor engagement (**FIG. 5B**). These data indicated that SIRPα-Fc-CD40L can stimulate CD40 signaling in the absence of cross-linking, possibly due to the chimeric protein's structure as a hexamer.

The murine version of the CD40/NFκB-luciferase system was established in CHOK1 cells. Like the human counterpart, the mSIRPα-Fc-CD40L chimeric protein consistently stimulated potent activation of the NFκB pathway, whereas a murine CD40 agonist antibody had no activity (**FIG. 5C**).

The observation that SIRPα-Fc-CD40L stimulated CD40 signaling prompted investigation of other cellular functions that depend upon CD40 signaling. CD8+ T cell-depleted PBMC were isolated from a total of 33-50 different human donors and cultured in the presence of a dose-titration of hSIRPα-Fc-CD40L (**FIG. 6A** and **FIG. 6B**). As compared to

a media-only negative control and the neoantigen Keyhole limpet haemocyanin (KLH) as positive control, soluble hSIRPα-Fc-CD40L was shown to stimulate dose-dependent proliferation of human PBMC over a 7-day culture (**FIG. 6A**). In addition, hSIRPα-Fc-CD40L was able to stimulate a dose-dependent increase in the number of IL-2-secreting PBMC on day 8 of the culture (**FIG. 6B)**.

Example 3: Visualization of tumor cells undergoing phagocytosis

Here, confocal microscopy can be used to visualize tumor cells undergoing phagocytosis by antigen-presenting cells (*e.g.,* macrophages and dendritic cells). Here, a combination of SIRPα(CD172a)-Fc-CD40L chimeric protein and an antibody-dependent cellular cytotoxicity-related antibody (*e.g.*, an anti-CD20 antibody) can stimulate phagocytosis of tumor cells by antigen-presenting cells. **FIG. 7A** shows macrophages that are fluorescently labeled with CD11b (**FIG. 7A**) and tumor cells that are fluorescently labeled with a FITC stain (**FIG. 7B**). **FIG. 7C** to **FIG. 7E** each show a confocal microscopy image of fluorescent markers for tumor cells (FITC stain). **FIG. 7F** to **FIG. 7H** each show a confocal microscopy image of fluorescent markers for tumor cells (FITC stain, **FIG. 7F**), macrophages (DAPI stain, **FIG. 7G**) and macrophages (DAPI stain, stitched image, **FIG. 7H**). By combining confocal images of the multiple fluorescent channels, phagocytosis promoted by the combination of the chimeric protein and the antibody can be visualized.

Example 4: Functional anti-tumor activity of the SIRPα(CD172a)-Fc-CD40L chimeric protein in combination with an anti-CD20 antibody

The *in vitro* ability of the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and an anti-CD20 antibody (Rituximab) to simulate/activate macrophage engulfment of tumor cells was determined. To this end, *in vitro* tumor cell phagocytosis assays were established to determine whether SIRPα-Fc-CD40L enhanced macrophage-mediated phagocytosis of various tumor cell lines both alone and in combination with anti-CD20 antibodies.

Initially, a variety of CD20+ lymphoma (Toledo, Raji, and Ramos) cell lines were co-cultured with human monocyte-derived macrophages to assay the amount of phagocytosis promoted by the SIRPα(CD172a)-Fc-CD40L chimeric protein in the presence or absence of an anti-CD20 antibody.

Co-cultures of Raji cells (a human Burkitt lymphoma tumor cell line) and macrophages were treated with control IgGs, an anti-CD20 antibody (Rituximab), the SIRPα(CD172a)-Fc-CD40L chimeric protein, or a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody. As shown in **FIG. 8A**, the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody was most effective in simulating/activating macrophage engulfment of tumor cells. This effectiveness in tumor engulfment was quantified and, as shown in **FIG. 8B**, the rate of tumor cells engulfed my macrophages in cultures treated with the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody was greater than the sum of the SIRPα(CD172a)-Fc-CD40L chimeric protein-alone treatments and the anti-CD20 antibody-alone treatments. Such synergistic effect was unexpected.

As shown in **FIG. 8C** (top left panel), RNA expression of the type I interferon regulatory gene INFα1 in Raji cells was significantly greater in cells treated with the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody than cells treated with either component alone. Similarly, the RNA expression of type I interferon regulatory gene IFNβ1 in cells treated with the combination was greater than the sum of the SIRPα(CD172a)-Fc-CD40L chimeric protein-alone treatments and the anti-CD20 antibody-alone treatments (**FIG. 8C,** bottom left panel). IFNβ protein synthesis was significantly greater in the cells treated with the combination (**FIG. 8C,** bottom right panel). Finally, unlike the untreated cells, cells treated with the combination increased the amount of phosphorylated IRF3 over time, even though the actual amounts of IRF3 and cGAS did not change (**FIG. 8C,** top right panel).

RNA was prepared from macrophages isolated using fluorescent activated cell sorting (FACS) from SIRPα-Fc-CD40L/Rituximab treated co-cultures macrophages and Toledo lymphoma cells. The RNA was assessed by qRT-PCR for expression of IFNα1 and IFNβ1, and the macrophage activation markers CD80 and CD86. As shown in **FIG. 8D,** monotherapies of the SIRPα-Fc-CD40L chimeric protein or of rituximab (an anti-CD20 antibody) induced macrophage activation and the expression of type I interferon genes in the isolated macrophages. Surprisingly, induction of these type I interferon genes was enhanced when macrophages were contacted with a combination of the SIRPα-Fc-CD40L chimeric protein and rituximab (**FIG. 8D**).

Co-cultures of Raji cells and macrophages that were stimulated with a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and an anti-CD20 antibody had the greatest phagocytosis activity among treatment groups, when measured by flow cytometry (**FIG. 8E**).

Against Toledo lymphoma cells, the phagocytosis-stimulating activity of SIRPα-Fc-CD40L/rituximab combinations on macrophages was partially inhibited when the macrophages' Fc receptors were blocked ("Fc Block") before addition of the SIRPα-Fc-CD40L chimeric protein and rituximab (**FIG. 8F**). In contrast, phagocytosis-stimulating activity of the combination was not impacted when macrophages were pre-treated with a CD40-blocking antibody ("CD40 Block"). Calreticulin on tumor cells has been shown to serve as a pro-phagocytic signal that facilitates tumor cell phagocytosis following blockade of the CD47/SIRPα pathway (Chao *et al.*, "Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47". Sci Transl Med. 2010;2(63):63ra94, the contents of which is incorporated by reference in its entirety). Although a calreticulin blocking peptide ("CALR Block") was unable to reduce the phagocytosis-stimulating activity of the combination, when the calreticulin blocking peptide and Fc receptor block were administered together, the phagocytosis-stimulating activity of the combination was greatly reduced (**FIG. 8F**). These data suggest that engagement of both calreticulin and the Fc receptor is required for efficient phagocytosis of CD20+ B cell lymphoma cells by the combination of the SIRPα-Fc-CD40L chimeric protein and rituximab.

Additionally, a macrophage reporter system was used to detect activation. Here, murine RAW264.7 cells were stably transfected with an interferon regulatory factor (IRF)-inducible reporter system, which comprised five IFN-stimulated response elements (ISG reporter, InvivoGen) (**FIG. 8G**). RAW264.7-ISG cells were co-cultured with murine A20

lymphoma cells in the presence or absence of murine SIRPα-Fc-CD40L and other control agents. After 24 hours, culture supernatant was collected and assessed for luciferase activity, which would indicate activation of a type I interferon response in the RAW-ISG reporter cells. As shown in **FIG.** 8G, a monotherapy with the SIRPα-Fc-CD40L chimeric protein stimulates an increase in interferon gene-driven luciferase activity. Commercially available recombinant murine Fc-CD40L was also able to stimulate IFN production. However, no significant signal was observed using a recombinant single-sided SIRPα-Fc protein, indicating that type I interferon activation acts downstream and independent from tumor cell phagocytosis, perhaps, through CD40 engagement. A monotherapy using the mouse rituximab surrogate (an anti-mouse CD20 antibody) induced a moderate IFN response. Surprisingly, the combination of the mSIRPα-Fc-CD40L chimeric protein and the rituximab surrogate had significantly amplified signal relative to the monotherapies (**FIG. 8G**). These data suggest that the combination of SIRPα-Fc-CD40L with targeted antibody-dependent cellular cytotoxicity (ADCC)/antibody-dependent cellular phagocytosis (ADCP)-competent antibodies may provide increased interferon expression relative to monotherapies. In other words, these combinations are able to increase tumor cell phagocytosis and initiate cellular pathways capable of activating antigen-presenting cells (APC), thereby, enhancing antigen processing/presentation. Additionally, these data indicate that the magnitude of the type I IFN response is greatly enhanced when the extracellular domain of SIRPα and the extracellular domain of CD40L are physically linked (as in the SIRPα-Fc-CD40L chimeric protein) as opposed to being provided as separate, individual proteins.

Finally, **FIG. 8H** shows a murine phagocytosis assay using bone marrow derived macrophages (BMDMs) co-cultured with A20 lymphoma or WEHI3 leukemia cells, in the presence of the mSIRPα-Fc-CD40L chimeric proteins or in the presence of an anti-CD47 antibody. CD47 is the ligand for SIRPα. Here, the mSIRPα-Fc-CD40L chimeric protein induced strong phagocytic activity in co-cultures of BDMDMs with either A20 or WEHI3 cells. Notably, the mSIRPα-Fc-CD40L chimeric protein induced a higher phagocytosis index than the CD47 blocking antibody.

Example 5: in vivo *dendritic cell activation by the SIRPα-Fc-CD40L chimeric protein*

An *in vivo* assay to examine SIRPα/CD47 function in mice was describe in Yi *et al.,* "Splenic Dendritic Cells Survey Red Blood Cells for Missing Self-CD47 to Trigger Adaptive Immune Responses. Immunity. 2015;43(4):764-75) (the contents of which is incorporated by reference in its entirety). This assay detects the activation status of splenic dendritic cells in response to SIRPα/CD47 inhibitors or to infused sheep red blood cells (RBC).

In these experiments, mice were treated with a single IV dose of sheep RBCs ($10x10^6$ cells; as a positive control), to a CD47 blocking antibody and a SIRPα blocking antibody (100 µg each), or the SIRPα-Fc-CD40L chimeric protein (at 100 or 300 µg). After 6 or 24 hours, mice were euthanized and their spleens excised, dissociated, and assessed by flow cytometry for populations of activated CD4+ dendritic cells (DCs) (**FIG. 9A,** left panel) or CD8+ dendritic cells (**FIG. 9A,** right panel); both populations of dendritic cells were also positive for MHC II (I-Ab), CD11c, and DC1R2. As shown

in **FIG. 9A**, intravenous administration of sheep RBCs, CD47 blocking antibodies, or SIRPα blocking antibodies all stimulated upregulation of both activated CD4+ and CD8α+ DCs that were positive for MHC II within 6 hours; however, administration of the murine SIRPα-Fc-CD40L chimeric protein greatly upregulated splenic CD4+ and CD8α+ DCs that expressed high levels of MHCII, CD80, and CD86, especially at the 24 hour time point. As shown in **FIG. 9B,** the SIRPα-Fc-CD40L chimeric protein induced a higher proportion of overall splenic DCs than was observed in the antibody treated groups.

Example 6: Analysis of combinations including the SIRPα(CD172a)-Fc-CD40L chimeric protein to stimulate phagocytosis

The *in vitro* ability of the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and an anti-CD20 antibody (Rituximab) to simulate phagocytosis of human tumor cells was determined.

Antibody-dependent cellular cytotoxicity (ADCC) competent antibodies (*e.g.,* an anti-CD20 antibody (Rituximab)) have been shown to stimulate tumor cell phagocytosis through the simultaneous binding of the target (*e.g.,* CD20 on malignant B cell) and Fc cross-linking of the antibody to antigen-presenting cells (APCs; *e.g.,* macrophages and dendritic cells).

These experiments used an *in vitro* phagocytosis assay with human donor macrophages and several human tumor cell lines (*e.g.,* Raji cells, a human Burkitt lymphoma tumor cell line), to test whether SIRPα-Fc-CD40L's blockade of the tumor cell antigen CD47 ("don't eat me signal") could stimulate phagocytosis, and potentially synergize with the anti-CD20 antibody, Rituximab. **FIG. 10A** is a cartoon illustrating steps used in this assay. In this assay, the tumor cells are tagged with a fluorescent marker which allows visualization of their engulfment (phagocytosis) by macrophages when CD47 is blocked by the SIRPα-Fc-CD40L chimeric protein or by the anti-CD47 antibody.

Co-cultures of tagged Raji cells and macrophages were treated with a variety of chimeric protein and antibody combinations, including control IgGs with an anti-CD20 antibody (Rituximab), an anti-CD47 antibody (CC9, Celgene), the SIRPα(CD172a)-Fc-CD40L chimeric protein, and/or pembrolizumab (an anti-PD-1 antibody; KEYTRUDA/MK 3475, Merck). As shown in **FIG. 10B** and **FIG. 10C**, the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody was effective in simulating phagocytosis of human tumor cells, especially when the anti-CD20 antibody is an IgG1 isotype. Such synergistic effect was unexpected.

This synergy was lost when Fc receptors on the macrophages were pre-blocked, but the stimulation of phagocytosis remained the same if CD40 was pre-blocked (**FIG. 11**), indicating that the extracellular domain of SIRPα on the SIRPα(CD172a)-Fc-CD40L chimeric protein is contributing to this mechanism of action.

Example 7: Mechanistic assays support in vitro *phagocytosis assay findings*

After a macrophage engulfs a tumor cell, the tumor cell begins to degrade/deteriorate inside the macrophage. Also, the macrophage activates signaling pathways, such as type I interferons (*e.g.*, IFNα and IFNβ), and the Stimulator of interferon genes (STING) associated signaling pathway.

In these experiments, mechanistic assays were undertaken to support the findings from the *in vitro* human tumor cell phagocytosis assays shown in **FIG. 10B, FIG. 10C,** and **FIG. 11**.

FIG. 12A and **FIG. 12B** show an IFNα (**FIG. 12A**) and IFNβ (**FIG. 12B**) ELISA on 24 hour macrophage:tumor cell co-cultures. In these figures, the term "ARC" refers to the SIRPα(CD172a)-Fc-CD40L chimeric protein. The mechanistic assays shown in **FIG. 12A** and **FIG. 12B** support the *in vitro* phagocytosis experimental findings of this example.

Example 8: Combinations of the SIRPα(CD172a)-Fc-CD40L chimeric protein and an anti-EGFR antibody or an anti-Her2 antibody simulate/activate macrophage engulfment of tumor cells

The *in vitro* ability of the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and an anti-EGFR antibody (cetuximab) or an anti-Her2 antibody (trastuzumab) to simulate/activate macrophage engulfment of tumor cells was determined.

The above-disclosed synergistic phagocytosis activity of the SIRPα-Fc-CD40L chimeric protein in combination with an antibody-dependent cellular phagocytosis (ADCP)-competent antibody was further examined in a variety of human tumor cell lines and using several ADCP targeted antibodies. In particular, EGFR+ melanoma (A431) cells and lung adenocarcinoma (HCC827) cells, EGFR- chronic myeloid leukemia (K562) cells, and HER2+ breast cancer (HCC1954HER2 HI and MCF7HER2 LOW) cells were used to facilitate combinations with an anti-EGFR antibody (cetuximab) and an anti-HER2 antibody (trastuzumab). Consistent with the lymphoma cell lines, disclosed above, monotherapy with the SIRPα-Fc-CD40L chimeric protein stimulated macrophage phagocytosis of tumor cells; this activity was enhanced when the SIRPα-Fc-CD40L chimeric protein was combined with the anti-EGFR antibody (**FIG. 13A** to **FIG. 13C**) or the anti-HER2 antibody (**FIG. 14A** and **FIG. 14B**).

As shown in **FIG. 13A** to **FIG. 13C**, co-cultures of various tumor cells: a high EGFR-expressing skin cancer cell line (A431), a high EGFR-expressing lung cancer cell line (HCC827), and a low EGFR-expressing Chronic myeloid leukemia (CML) cell line (K562)) and macrophages were treated with control IgGs, an anti-EGFR antibody (cetuximab), the SIRPα(CD172a)-Fc-CD40L chimeric protein, or a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-EGFR antibody. The data shown in **FIG. 13A** and **FIG. 13B** versus the data shown in **FIG. 13C**, demonstrates that the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-EGFR antibody was more effective in simulating/activating macrophage engulfment of high EGFR-expressing tumor cells than the combination was in simulating/activating macrophage engulfment of low EGFR-expressing tumor cells. Similar to other

reports which use K562 cells as a negative control cell line for phagocytosis, no phagocytic activity was observed with monotherapy or combinations of SIRPα-Fc-CD40L with cetuximab (**FIG. 13C**).

As shown in **FIG. 14A** and **FIG. 14B**, co-cultures of a high Her2-expressing breast cancer cell line (HCC1954) or a low Her2-expressing breast cancer cell line (MCF7) and macrophages were treated with control IgGs, an anti-Her2 antibody (trastuzumab), the SIRPα(CD172a)-Fc-CD40L chimeric protein, or a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-EGFR antibody. The data shown in **FIG. 14A** versus the data shown in **FIG. 14B**, demonstrates that the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-Her2 antibody was more effective in simulating/activating macrophage engulfment of high Her2-expressing tumor cells than the combination was in simulating/activating macrophage engulfment of low Her2-expressing tumor cells. Interestingly, trastuzumab did not induce phagocytosis in the HER2LOW cell line MCF7 (**FIG. 14B**), whereas the SIRPα-Fc-CD40L chimeric protein demonstrated monotherapy activity.

Example 8: Functional in vivo *anti-tumor activity of specific combinations of antibodies directed to immune checkpoint molecules and chimeric proteins*

The *in vivo* ability of specific combinations of antibodies directed to immune checkpoint molecules and chimeric proteins to target and reduce tumor volume was determined.

BALB/C mice were inoculated with 500,000 CT26 tumor cells. Eight days after inoculation, there was no significant difference between starting tumor volumes among the mice, *i.e.,* volumes were approximately 100 mm^3. Eight days after inoculation, treatment began according to the schedule shown in **FIG. 15A**. In **FIG. 15B**, the treatments included: an anti-CTLA-4 antibody (9D9), an anti-PD-1 antibody (RMP1-14), an anti-OX40 antibody (OX86), or the SIRPα(CD172a)-Fc-CD40L chimeric protein. In **FIG. 15C**, the treatments included: the anti-CTLA-4 antibody then the anti-PD1 antibody, the anti-CTLA-4 antibody then the anti-OX40 antibody, and the anti-CTLA-4 antibody then the SIRPα(CD172a)-Fc-CD40L chimeric protein. In **FIG. 15D**, the treatments included the SIRPα(CD172a)-Fc-CD40L chimeric protein then the anti-CTLA-4 antibody. Tumor sizes were assayed every other day until the 27th day after inoculation. Mice that rejected the tumor were re-challenged with a secondary tumor (300,000 CT26 tumor cells) on the opposing flank, and primary/secondary tumors continued to be measured.

As shown in the final column of **FIG. 15A**, relative to vehicle, all treatments were effective in promoting survival of tumor-bearing mice.

As shown in **FIG. 15B**, relative to vehicle, all single-component treatments were effective in reducing tumor volume. Likewise, as shown in **FIG. 15C** and **FIG. 15D**, the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CTLA-4 antibody showed reduced tumor volume over the course of the study. In particular, the most significant improvement was observed for treatments when the SIRPα(CD172a)-Fc-CD40L chimeric protein was administered after the anti-CTLA-4 antibody.

Example 9: Functional in vivo *anti-tumor activity of specific combinations of a STING agonist and chimeric proteins*

The *in vivo* ability of specific combinations of a stimulator of interferon genes (STING) agonist and chimeric proteins to target and reduce tumor volume was determined.

BALB/C mice were inoculated with 500,000 CT26 tumor cells. Eight days after inoculation, there was no significant difference between starting tumor volumes among the mice, *i.e.,* volumes were approximately 100 mm^3. Eight days after inoculation treatment began according to the schedule shown in **FIG. 15A**. In **FIG. 16A**, the treatments included: the STING agonist (DMXAA); an anti-PD-1 antibody (RMP1-14); an anti-OX40 antibody (OX86); or the SIRPα(CD172a)-Fc-CD40L chimeric protein. In **FIG. 16B**, the treatments included: DMXAA then the anti-PD1 antibody; DMXAA then the anti-OX40 antibody; and DMXAA then the SIRPα(CD172a)-Fc-CD40L chimeric protein. Tumor sizes were assayed every other day until the 27th day after inoculation. Mice that rejected the tumor were re-challenged with a secondary tumor (300,000 CT26 tumor cells) on the opposing flank, and primary/secondary tumors continued to be measured. In these experiments, DMXAA was administered intratumorally (IT) and the other agents were administered intraperitoneally (IP).

As shown in the final column of **FIG. 16A**, relative to vehicle, all treatments were effective in promoting survival of tumor-bearing mice.

As shown in **FIG. 16A**, relative to vehicle, all single-component treatments were effective in reducing tumor volume. Likewise, as shown in **FIG. 16B**, the combination treatments showed reduction in tumor volume over the course of the study.

Example 10: Functional in vivo *anti-tumor activity of specific combinations of antibodies directed to immune checkpoint molecules and the SIRPα(CD172a)-Fc-CD40L chimeric protein*

The *in vivo* ability of combinations of the SIRPα(CD172a)-Fc-CD40L chimeric protein with anti-CTLA-4 antibodies (**FIG. 17A** and **FIG. 17B**) or the SIRPα(CD172a)-Fc-CD40L chimeric protein with anti-PD-1 antibodies (**FIG. 18A** and **FIG. 18B**) to target and treat tumors was determined. **FIG. 17C** includes data relevant to the graphs of **FIG. 17A** and **FIG. 17B**. **FIG. 18C** includes data relevant to the graphs of **FIG. 18A** and **FIG. 18B**.

Mice were inoculated with tumors and were treated with a vehicle, an antibody, the SIRPα(CD172a)-Fc-CD40L chimeric protein, or combinations of the SIRPα(CD172a)-Fc-CD40L chimeric protein and an antibody; in the combinations, the SIRPα(CD172a)-Fc-CD40L chimeric protein was administered before the antibody, the SIRPα(CD172a)-Fc-CD40L chimeric protein was administered after the antibody, or the SIRPα(CD172a)-Fc-CD40L chimeric protein was administered with the antibody.

FIG. 17A shows changes in tumor size (*i.e.*, volume) resulting from treatments comprising the SIRPα(CD172a)-Fc-CD40L chimeric protein and/or the anti-CTLA-4 antibody. **FIG. 17B** shows Kaplan-Meier plots of the percent survival

days after tumor inoculation resulting from treatments comprising the SIRPα(CD172a)-Fc-CD40L chimeric protein and/or the anti-CTLA-4 antibody. Surprisingly, the administration order of antibody and chimeric protein affected the treatment outcome. More specifically, although the combinations of the chimeric protein and antibody provided an improved therapeutic benefit (compared to any of the chimeric protein alone treatment or the antibody alone treatments), among the combination treatments, the combination where the anti-CTLA-4 antibody was administered before the SIRPα(CD172a)-Fc-CD40L chimeric protein had the greatest treatment outcome whereas the combination where the SIRPα(CD172a)-Fc-CD40L chimeric protein was administered before the anti-CTLA-4 antibody had a less great treatment outcome.

FIG. 18A shows changes in tumor size (*i.e.*, volume) resulting from treatments comprising the SIRPα(CD172a)-Fc-CD40L chimeric protein and/or the anti-PD-1 antibody. **FIG. 18B** shows Kaplan-Meier plots of the percent survival days after tumor inoculation resulting from treatments comprising the SIRPα(CD172a)-Fc-CD40L chimeric protein and/or the anti-PD-1 antibody. Surprisingly, the administration order of antibody and chimeric protein affected the treatment outcome. More specifically, among the combination treatments, the combination where the anti-PD-1 antibody was administered with the SIRPα(CD172a)-Fc-CD40L chimeric protein had the greatest treatment outcome whereas the combination where the SIRPα(CD172a)-Fc-CD40L chimeric protein was administered before or after the anti-PD-1 antibody had a less great treatment outcome.

Interestingly, there was minimal improvement in tumor control (when compared to monotherapy) when treatment with mSIRPα-Fc-CD40L occurred before treatment with an anti-CTLA-4 antibody or an anti-PD1 antibody, or when the mSIRPα-Fc-CD40L treatment followed the anti-PD1 antibody treatment (**FIG. 17A** and **FIG. 18A**). Significant improvements in tumor control (including a number of complete rejections) and overall survival were observed when mSIRPα-Fc-CD40L was given together with anti-PD1 (43% rejection), together with anti-CTLA-4 (57% rejection), or following anti-CTLA-4 (60% rejection) (**FIG. 17A** and **FIG. 18A**,). Importantly, nearly all mice that rejected the primary CT26 tumor also rejected a secondary tumor challenge without additional treatments (**FIG. 17C** and **FIG. 18C**).

To assess the basis for synergy between PD1/CTLA-4 blockade and mSIRPα-Fc-CD40L, CT26 tumors were excised from mice 11 days after inoculation, and were then treated with either an anti-PD1 antibody (clone RMP1-14) or an anti-CTLA-4 antibody (clone 9D9). Interestingly, both agents expanded CD40+ dendritic cells/B cells and CD3+ T cells, and induced the up-regulation of MHC I and MHC II (**FIG. 19**). Thus, initial treatment with an anti-PD1 antibody or an anti-CTLA-4 antibody stimulated expansion of CD40-expressing immune cells. Without wishing to be bound by theory, this result may explain the subsequent increase in response when followed by treatment with SIRPα-Fc-CD40L. Interestingly, checkpoint blockade did not appear to affect the tumor cell's surface expression of CD47. This suggests that checkpoint combination synergy functions independent of phagocytosis activity.

The experimental evidence shows that treatments with the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CTLA-4 antibody or treatments with the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-PD-1 antibody

provide most significant improvements in tumor volume and survival relative to treatments with the SIRPα(CD172a)-Fc-CD40L chimeric protein alone or either antibody alone.

Example 11: Functional in vivo *anti-tumor activity of specific combinations of antibody-dependent cellular cytotoxicity (ADCC) competent antibodies and the SIRPα(CD172a)-Fc-CD40L chimeric protein*

The *in vivo* ability of the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and an anti-CD20 antibody to stimulated/activate tumor reduction was determined.

In these experiments, the syngeneic CT26 colon tumor model was used to provide an initial assessment of the anti-tumor activity of the SIRPα-Fc-CD40L chimeric protein in comparison to CD40 agonist and CD47 blocking antibodies. Implanted CT26 tumors were allowed to grow to ~30 mm^3 before treatment was initiated with a fixed regimen of two doses of either a CD40 agonist antibody (clone FGK4.5), a CD47 blocking antibody (clone MIAP301), a combination of the two antibodies, or the murine SIRPα-Fc-CD40L chimeric protein. As compared to vehicle controls, both the CD40 agonist and CD47 blocking antibodies provided moderate extensions in tumor growth, with no mice completely rejecting primary tumors in the CD40 agonist monotherapy group (**FIG. 20A**). Mice treated with a combination of CD40 and CD47 antibodies were observed to have a longer delay in tumor outgrowth and 33% of mice rejecting tumors. In comparison to the antibody groups, complete tumor rejection was observed in 62% of the mice treated with the mSIRPα-Fc-CD40L chimeric protein had significant prolongation in tumor growth and survival (Mantel-Cox test, p=0.0047 vs. anti-CD40/anti-CD47 combination group). Importantly, a majority of the mice, treated with the mSIRPα-Fc-CD40L chimeric protein, which rejected the primary tumor, were also able to reject a secondary tumor challenge in the absence of additional treatment with the SIRPα-Fc-CD40L chimeric protein (60%; **FIG. 20A**). In the mice treated with an antibody combination and the mice treated with the SIRPα-Fc-CD40L chimeric protein, there was an increase in the proportion of AH1-tetramer positive CD8+ T cells in both the tumor and spleen (**FIG. 20B**). Based on this observation, these experiments were repeated in the setting of antibody-mediated depletion of both CD4+ and CD8+ cells (**FIG. 20C**).

These later experiments confirmed that, while CD4+ T cells are partially required for a therapeutic benefit from the SIRPα-Fc-CD40L chimeric protein, the absence of CD8+ cells completely eliminated the therapeutic benefit obtained from the SIRPα-Fc-CD40L chimeric protein. CD4 and CD8 depletion was verified in the peripheral blood at multiple time points during the experiment (see, **FIG. 20F** and **FIG. 20G**).

Since the SIRPα-Fc-CD40L chimeric protein potentiated the activity of the anti-CD20 antibody rituximab, combination of the SIRPα-Fc-CD40L chimeric protein with a murine surrogate for rituximab: an anti-mouse CD20 antibody; clone AISB12) was investigated in two CD20-positive mouse tumor models, WEHI3 and A20. In both tumor models, a similar control of established tumor growth was observed when anti-CD20 antibodies or the SIRPα-Fc-CD40L chimeric protein was used in a monotherapy (**FIG. 20D** and **FIG. 20E**; in these figures, the SIRPα-Fc-CD40L chimeric protein is identified

as "ARC"). **FIG. 20D** shows *in vivo* changes of Warner myelomonocytic leukemia (WEH13) tumor size (*i.e.,* volume) resulting from treatments with an anti-CD20 antibody, the SIRPα(CD172a)-Fc-CD40L chimeric protein, and the SIRPα(CD172a)-Fc-CD40L chimeric protein in combination with the anti-CD20 antibody. The combination reduced tumor size greater than either component alone. In a second set of experiments, a combination of an anti-IFNAR-1 antibody and the anti-CD20 antibody, the SIRPα(CD172a)-Fc-CD40L chimeric protein in combination with the anti-IFNAR-1 antibody, and a triple combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein, the anti- IFNAR-1 antibody, and the anti-CD20 antibody were tested. Surprisingly, the benefit in tumor reduction resulting from treatments with the SIRPα(CD172a)-Fc-CD40L chimeric protein is negated by co-treatments with the anti-IFNAR-1 antibody. Indeed, the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-INFAR1 antibody provided less tumor reduction than treatments with the SIRPα(CD172a)-Fc-CD40L chimeric protein alone; the triple combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein, the anti-CD20 antibody, and the anti-INFAR1 antibody provided less tumor reduction than treatments with the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CD20 antibody. As mentioned above, in mice with established WEHI3 tumors, antibody-mediated blockade of IFNAR1 strongly reduced the efficacy of the mSIRPα-Fc-CD40L chimeric protein both alone and in combination with the anti-CD20 antibody (**FIG. 20D** and **FIG. 20E**). IFN alpha-receptor blockade most significantly impacted the mice treated with the SIRPα-Fc-CD40L chimeric protein and less of an effect on mice treated with an anti-CD20 antibody monotherapy. Consistent with these observations, tumor control was similar between the anti-CD20 antibody monotherapy and the combination the SIRPα-Fc-CD40L chimeric protein and the anti-CD20 antibody. This suggest that a majority of the combinatorial benefit requires a functional type I interferon response. The depletion of IFNAR1+ cells was confirmed at multiple time points in the peripheral blood by flow cytometry (**FIG. 20H**).

These data suggest that IFNAR-1 and its related pathways are relevant to the anti-tumor effects produced by the SIRPα(CD172a)-Fc-CD40L chimeric protein and combinations of the SIRPα(CD172a)-Fc-CD40L chimeric protein with an anti-cancer antibody.

Example 12: Safety and activity of SIRPα-Fc-CD40L in non-human primates

Clinical utility of SIRPα/CD47 inhibition is tempered by expression of CD47 on erythrocytes and platelets, and the associated risk of hemolysis and thrombocytopenia observed with some agents (Lin *et al.* "TTI-621 (SIRPαIphaFc), a CD47-blocking cancer immunotherapeutic, triggers phagocytosis of lymphoma cells by multiple polarized macrophage subsets. PLoS One. 2017;12(10); Advani et al. CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. N Engl J Med. 2018;379(18):1711-21; the contents of each of which is incorporated by reference in its entirety).

The Fc domain of the SIRPα-Fc-CD40L chimeric protein does not bind effector Fc receptors (**FIG. 3E**), and *in vitro* studies did not reveal evidence of hemolysis in human or cynomolgus macaque erythrocytes (see, **FIG. 21**, **FIG. 22A,**

and **FIG. 22B**). However, the *in vitro* systems used to test this question have significant limitations, including a complete lack of macrophages in the test system. Due to high homology of CD47 between human and cynomolgus macaque (98.69% identity), the cynomolgus macaque was used to develop the priming dose strategy for the Hu5F9-G4 antibody due to the observation of hemolysis following a single dose, which demonstrates that cynomolgus macaques are a relevant species for evaluating this toxicity (Liu *et al.* "Pre-Clinical Development of a Humanized Anti-CD47 Antibody with Anti-Cancer Therapeutic Potential. PLoS One. 2015;10(9), the contents of which is incorporated by reference in its entirety).

The experiments of this example tested the safety and activity of the human SIRPα-Fc-CD40L chimeric protein following repeat doses in cynomolgus macaques. Briefly, naïve cynomolgus macaques were administered the human SIRPα-Fc-CD40L chimeric protein by intravenous infusion every week, for five consecutive weeks, at doses of 0.1, 1, 10 and 40 mg/kg. Standard hematology and clinical chemistry parameters were collected before and after each dose. There was no evidence of hemolysis or thrombocytopenia as a result of treatment with the human SIRPα(CD172a)-Fc-CD40L chimeric protein over the course of the study (**FIG. 21**).

Mild declines in hematology parameters were noted, however these declines were noted in the vehicle control group and were therefore most likely related to procedural effects and repeated blood collections. Episodic fluctuation was observed in the total number of lymphocytes before and after each dose, which did not depart from the upper and lower range of normal in cynomolgus macaques. Receptor occupancy was evaluated on circulating CD40+ lymphocytes. Lymphocyte fluctuations occurred in parallel with a dose-dependent decrease in the number of CD40+ cells in the peripheral blood, which may reflect migration of those cells into peripheral tissues (**FIG. 21**, bottom right). This peripheral decrease in CD40+ B cells is consistent with similar observations seen in the blood of mice treated with mSIRPα-Fc-CD40L (**FIG. 22C** and **FIG. 22D**). Interestingly, in mice, the decrease in B cells was accompanied by a significant increase in CD8+ dendritic cells. Lastly, a dose-dependent increases in multiple serum cytokines was observed following each infusion of SIRPα-Fc-CD40L, including multiple cytokines/chemokines, including CCL2, CXCL9, CXCL10, IL-6, IL-15, IL-17A and IL-23; together suggestive of on-target pharmacodynamic biology.

FIG. 23A to **FIG. 23C** are schematic illustrations showing proposed SIRPα-Fc-CD40L mechanisms of action.

Without wishing to be bound by theory, the SIRPα(CD172a)-Fc-CD40L chimeric proteins of the present invention and/or the SIRPα(CD172a)-Fc-CD40L chimeric proteins used in methods of the present invention may operate according to the following mechanisms. First, the SIRPα(CD172a)-Fc-CD40L chimeric proteins may directly activate antigen presenting cells by binding to CD40 on APCs. Here, an advantage may be antigen-specific CD8 stimulation and/or programming of immune memory. When used in a combination, antibodies related to checkpoint molecules may increase CD40 target density for SIRPα(CD172a)-Fc-CD40L costimuation and upregulation of antigen presentation machinery. Second, the SIRPα(CD172a)-Fc-CD40L chimeric proteins may directly block CD47 inhibition by tumor cells blocking and sequestering CD47 on tumor cells. Here, an advantage may be enhanced tumor phagocytosis and

increased antigen cross-presentation. . When used in a combination, antibody-dependent cellular cytotoxicity-related antibodies increase targeted tumor phagocytosis, antigen cross-presentation and anti-tumor response.

Example 13: Production and characterization of SIRPα-Fc-OX40L

The extracellular domain (ECD) of SIRPα and the ECD of OX40L were fused *via* an antibody Fc domain to generate SIRPα-Fc-OX40L chimeric proteins. Mammalian cells were then transfected with the mSIRPα-Fc-OX40L expressing construct, and the secreted protein was purified from conditioned media by affinity chromatography. The purified protein was then analyzed for the presence of each individual domain by Western blotting using anti-SIRPα, anti-Fc, and anti-OX40L antibodies (**FIG. 24**). These blots revealed a glycosylated protein that formed a dimer under non-reducing conditions by SDS-PAGE. The reduced and deglycosylated form of the protein migrated at the predicted monomeric molecular weight.

The SIRPα-Fc-OX40L chimeric proteins were characterized using assays as described above for the SIRPα-Fc-CD40L chimeric proteins.

Example 14: Functional in vivo *anti-tumor activity of specific combinations of antibodies directed to immune checkpoint molecules and the SIRPα-Fc-OX40L chimeric protein*

The *in vivo* ability of combinations of the SIRPα-Fc-OX40L chimeric protein with anti-CTLA-4 antibodies or the SIRPα-Fc-OX40L chimeric protein with anti-PD-1 antibodies to target and treat tumors was determined. (**FIG. 25A** and **FIG. 25B**) **FIG. 25C** includes data relevant to the graphs of **FIG. 25A** and **FIG. 25B**.

Mice were inoculated with tumors and were treated with a vehicle, the anti-PD-1 antibody, the anti-CTLA-4 antibody, a SIRPα-Fc fusion protein, the Fc-OX40L fusion protein, a combination of the SIRPα-Fc fusion protein and the Fc-OX40L fusion protein, the SIRPα(CD172a)-Fc-OX40L chimeric protein, a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and anti-PD-1 antibody, or a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CTLA-4 antibody.

FIG. 25A shows changes in tumor size (*i.e.*, volume) resulting from above-listed treatments. As shown, each of the SIRPα(CD172a)-Fc-OX40L chimeric protein, the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and anti-PD-1 antibody, and the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CTLA-4 antibody were effective in reducing tumor size. **FIG. 25B** shows Kaplan-Meier plots of the percent survival days after tumor inoculation resulting from the above listed treatments. Only mice treated with the SIRPα(CD172a)-Fc-OX40L chimeric protein, the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and anti-PD-1 antibody, or the combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and the anti-CTLA-4 antibody had any survivors by the sixteenth day following treatment. Importantly, only the mice treated with a combination of the SIRPα(CD172a)-Fc-

CD40L chimeric protein and antibody were able to reject primary tumor and none of the other treatment groups were able to reject the primary tumor. (**FIG. 25C**).

Example 15: Functional in vivo *anti-tumor activity of specific combinations of antibodies directed to immune checkpoint molecules and the SIRPα-Fc-LIGHT chimeric protein*

The *in vivo* ability of combinations of the SIRPα-Fc-LIGHT chimeric protein with anti-PD-1 antibodies to target and treat tumors was determined. (**FIG. 26A** and **FIG. 26B**) **FIG. 26C** and **FIG. 26D** include data relevant to the graphs of **FIG. 26A** and **FIG. 26B**.

Mice were inoculated with tumors and were treated with a vehicle, the anti-PD-1 antibody, the SIRPα(CD172a)-Fc-LIGHT chimeric protein, or a combination of the SIRPα(CD172a)-Fc-LIGHT chimeric protein and anti-PD-1 antibody.

FIG. 26A shows changes in tumor size (*i.e.*, volume) resulting from above-listed treatments. As shown, the combination of the SIRPα(CD172a)-Fc-LIGHT chimeric protein and the anti-PD-1 antibody was the most effective in reducing tumor size. **FIG. 26B** shows Kaplan-Meier plots of the percent survival days after tumor inoculation resulting from the above listed treatments. Only mice treated with the combination of the SIRPα(CD172a)-Fc-LIGHT chimeric protein and anti-PD-1 antibody had any survivors by the twenty-fifth day following treatment. Importantly, only the mice treated with a combination of the SIRPα(CD172a)-Fc-CD40L chimeric protein and anti-PD-1 antibody were able to reject primary tumor whereas none of the other treatment groups were able to reject the primary tumor (**FIG. 26D**). Moreover, mice which received the combination were also able to reject a secondary tumor challenge in the absence of additional treatment with the SIRPα-Fc-LIGHT chimeric protein (66.6%; **FIG. 26D**).

In any of the above-described Examples, the therapeutic activity of the treatments may further be assayed. In particular, changes in pharmacodynamic biomarkers showing tumor rejection will be determined by cytokine elevations in serum (*in vivo)* or changes in pharmacodynamic biomarkers *in vitro* in immune-related cells incubated with the super-antigen Staphylococcal enterotoxin B (SEB assay) or when cultured in AIM V media will be determined. Exemplary pharmacodynamic biomarkers include IFNγ, IL-2, IL-4, IL-5, IL-6, and IL-17A.

INCORPORATION BY REFERENCE

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

Specifically, additional teachings related to the present invention are found, in one or more of WO2018/157162; WO2018/157165; WO2018/157164; WO2018/157163; and WO2017/059168, the contents of each of which is incorporated herein by reference in its entirety.

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

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

EQUIVALENTS

While the invention has been disclosed in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

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

CLAIMS

What is claimed is:

1. A method for treating a cancer in a subject in need thereof comprising:

providing the subject a first pharmaceutical composition comprising a heterologous chimeric protein comprising:

(a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand,

(b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and

(c) a linker linking the first domain and the second domain; and

providing the subject a second pharmaceutical composition comprising an antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), PD-1, or CTLA-4 and/or capable of, respectively, inhibiting the interaction of CD20, EGFR, Her2, PD-1, or CTLA-4 with one or more of its ligands.

2. The method of claim 1, wherein the first pharmaceutical composition and the second pharmaceutical composition are provided simultaneously.

3. The method of claim 1, wherein the first pharmaceutical composition is provided after the second pharmaceutical composition is provided.

4. The method of claim 1, wherein the first pharmaceutical composition is provided before the second pharmaceutical composition is provided.

5. The method of any one of claims 1 to 3, wherein the dose of the first pharmaceutical composition is less than the dose of the first pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the second pharmaceutical composition.

6. The method of any one of claims 1, 2, or 4, wherein the dose of the second pharmaceutical composition provided is less than the dose of the second pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the first pharmaceutical composition.

7. The method of any one of claims 1 to 6, wherein the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the first pharmaceutical composition.

8. The method of any one of claims 1 to 7, wherein the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the second pharmaceutical composition.

9. A method for treating a cancer in a subject comprising:

providing the subject a pharmaceutical composition comprising a heterologous chimeric protein comprising:

(a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand,

(b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and

(c) a linker linking the first domain and the second domain;

wherein the subject has undergone or is undergoing treatment with an antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), PD-1, or CTLA-4 and/or capable of, respectively, inhibiting the interaction of CD20, EGFR, Her2, PD-1, or CTLA-4 with one or more of its ligands.

10. The method of claim 9, wherein the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with an antibody that is capable of binding CD20, EGFR, or Her2.

11. A method for treating a cancer in a subject comprising:

providing the subject a pharmaceutical composition comprising antibody that is capable of binding CD20, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), PD-1, or CTLA-4 and/or capable of, respectively, inhibiting the interaction of CD20, EGFR, Her2, PD-1, or CTLA-4 with one or more of its ligands;

wherein the subject has undergone or is undergoing treatment with a heterologous chimeric protein comprising:

(a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand,

(b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, a portion of the extracellular domain of OX40L, wherein the portion is capable of binding an OX40L receptor, or a portion of the extracellular domain of LIGHT, wherein the portion is capable of binding a LIGHT receptor, and

(c) a linker linking the first domain and the second domain.

12. The method of claim 11, wherein the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with the heterologous chimeric protein.

13. The method of any one of claims 1 to 12, wherein the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L, OX40L, or LIGHT.

14. The method of any one of claims 1 to 13 wherein the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

15. The method of any one of claims 1 to 14, wherein the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain.

16. The method of claim 15, wherein the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG1 or IgG4, *e.g.,* human IgG4 or human IgG4.

17. The method of claim 15 or claim 16, wherein the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

18. The method of any one of claims 1 to 17 wherein the heterologous chimeric protein comprises:

(a) a first domain comprising a portion of SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, OX40L, or LIGHT, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

19. The method of any one of claims 1 to 18, wherein the antibody capable of binding CD20 is selected from rituximab, obinutuzumab, ofatumumab, ocrelizumab, ocaratuzumab, and veltuzumab.

20. The method of claim 19, wherein the antibody capable of binding CD20 is rituximab.

21. The method of any one of claims 1 to 18, wherein the antibody capable of binding EGFR is selected from cetuximab, ABP 494 (Actavis), CT-P15 (Celltrion), STI-001 (Sorrento), panitumumab, necitumumab, nimotuzumab, matuzumab, and chimeric 806 (ch806).

22. The method of claim 21, wherein the antibody capable of binding EGFR is cetuximab.

23. The method of any one of claims 1 to 18, wherein the antibody capable of binding HER2 is selected from trastuzumab, trastuzumab deruxtecan, ado-trastuzumab emtansine (T-DM1), trastuzumab-pkrb, trastuzumab-dkst, pertuzumab, margetuximab, PRS343, and ARX788.

24. The method of claim 23, wherein the antibody capable of binding HER2 is trastuzumab.

25. The method of any one of claims 1 to 18, wherein the antibody that is capable of binding CTLA-4 is selected from the group consisting of YERVOY (ipilimumab), 9D9, tremelimumab (formerly ticilimumab, CP-675,206; MedImmune), AGEN1884, and RG2077.

26. The method of any one of claims 1 to 18, wherein the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

27. The method of any one of claims 1 to 26, wherein the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

28. The method of any one of claims 1 to 27, wherein the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

29. The method of any one of claims 1 to 28, wherein the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

30. The method of claim 28 or claim 29, wherein the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

31. A method for treating a cancer in a subject in need thereof comprising:

providing the subject a first pharmaceutical composition comprising a stimulator of interferon genes (STING) agonist, and

providing the subject a second pharmaceutical composition comprising a heterologous chimeric protein comprising:

(a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand,

(b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and

(c) a linker linking the first domain and the second domain.

32. The method of claim 31, wherein the first pharmaceutical composition and the second pharmaceutical composition are provided simultaneously.

33. The method of claim 31, wherein the first pharmaceutical composition is provided after the second pharmaceutical composition is provided.

34. The method of claim 31, wherein the first pharmaceutical composition is provided before the second pharmaceutical composition is provided.

35. The method of any one of claims 31 to 34, wherein the dose of the first pharmaceutical composition is less than the dose of the first pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the second pharmaceutical composition.

36. The method of any one of claims 31, 32, or 34, wherein the dose of the second pharmaceutical composition provided is less than the dose of the second pharmaceutical composition provided to a subject who has not undergone or is not undergoing treatment with the first pharmaceutical composition.

37. The method of any one of claims 31 to 36, wherein the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the first pharmaceutical composition.

38. The method of any one of claims 31 to 37, wherein the subject has an increased chance of survival, without gastrointestinal inflammation and weight loss, and/or a reduction in tumor size or cancer prevalence when compared to a subject who has only undergone or is only undergoing treatment with the second pharmaceutical composition.

39. A method for treating a cancer in a subject comprising:

providing the subject a pharmaceutical composition comprising a heterologous chimeric protein comprising:

(a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand,

(b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and

(c) a linker linking the first domain and the second domain,

wherein the subject has undergone or is undergoing treatment with a stimulator of interferon genes (STING) agonist.

40. The method of claim 39, wherein the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with a STING agonist.

41. A method for treating a cancer in a subject comprising:

providing the subject a pharmaceutical composition comprising a stimulator of interferon genes (STING) agonist,

wherein the subject has undergone or is undergoing treatment with a heterologous chimeric protein comprising:

(a) a first domain comprising a portion of the extracellular domain of SIRPα(CD172a), wherein the portion is capable of binding a SIRPα(CD172a) ligand,

(b) a second domain comprising a portion of the extracellular domain of CD40L, wherein the portion is capable of binding a CD40L receptor, and

(c) a linker linking the first domain and the second domain.

42. The method of claim 41, wherein the dose of the pharmaceutical composition provided to the subject is less than the dose of the pharmaceutical composition that is provided to a subject who has not undergone or is not undergoing treatment with the heterologous chimeric protein.

43. The method of any one of claims 31 to 42, wherein the heterologous chimeric protein comprises a first domain which comprises substantially the entire extracellular domain of SIRPα(CD172a) and/or a second domain which comprises substantially the entire extracellular domain of CD40L.

44. The method of any one of claims 31 to 43, wherein the linker is a polypeptide selected from a flexible amino acid sequence, an IgG hinge region, and an antibody sequence.

45. The method of any one of claims 31 to 44, wherein the linker comprises at least one cysteine residue capable of forming a disulfide bond and/or comprises a hinge-CH2-CH3 Fc domain.

46. The method of claim 45, wherein the linker comprises a hinge-CH2-CH3 Fc domain derived from IgG4, *e.g.,* human IgG4.

47. The method of claim 45 or claim 46, wherein the linker comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

48. The method of any one of claims 31 to 47, wherein the heterologous chimeric protein comprises:

(a) a first domain comprising a portion SIRPα(CD172a),

(b) a second domain comprising a portion of CD40L, and

(c) a linker comprising a hinge-CH2-CH3 Fc domain.

49. The method of any one of claims 31 to 48, wherein the STING agonist is selected from the group consisting of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), MIW815(ADU-S100), CRD5500, MK-1454, SB11285, or IMSA101.

50. The method of any one of claims 31 to 49, wherein the cancer is or is related to a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

51. The method of any one of claims 31 to 50, wherein the subject has a cancer that is poorly responsive or is refractory to treatment comprising an antibody that is capable of binding PD-1 or binding a PD-1 ligand.

52. The method of any one of claims 31 to 51, wherein the cancer is poorly responsive or is non-responsive to treatment with an antibody that is capable of binding PD-1 or binding a PD-1 ligand after 12 weeks or so of such treatment.

53. The method of claim 72 or claim 52, wherein the antibody that is capable of binding PD-1 or binding a PD-1 ligand is selected from the group consisting of nivolumab (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), RMP1-14, AGEN2034 (AGENUS), cemiplimab (REGN-2810), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), Ibrutinib (PHARMACYCLICS/ABBVIE), atezolizumab (TECENTRIQ, GENENTECH), and MPDL328OA (ROCHE).

FIG. 1A

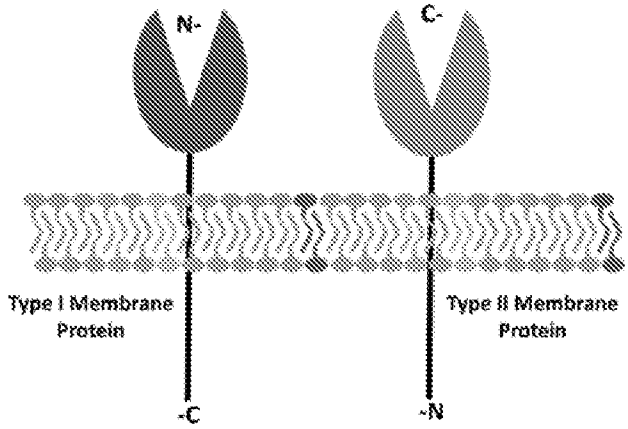


FIG. 1B

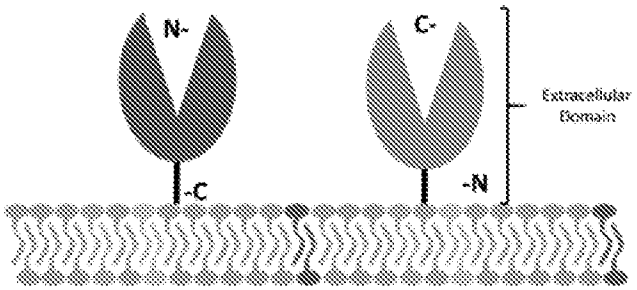


FIG. 1C

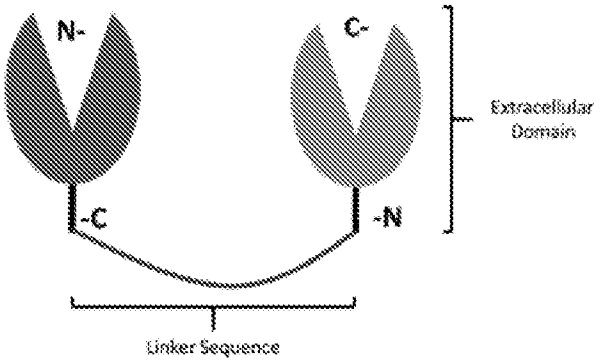


FIG. 1D

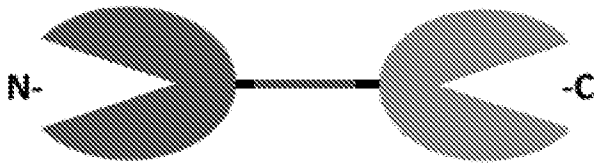


FIG. 2

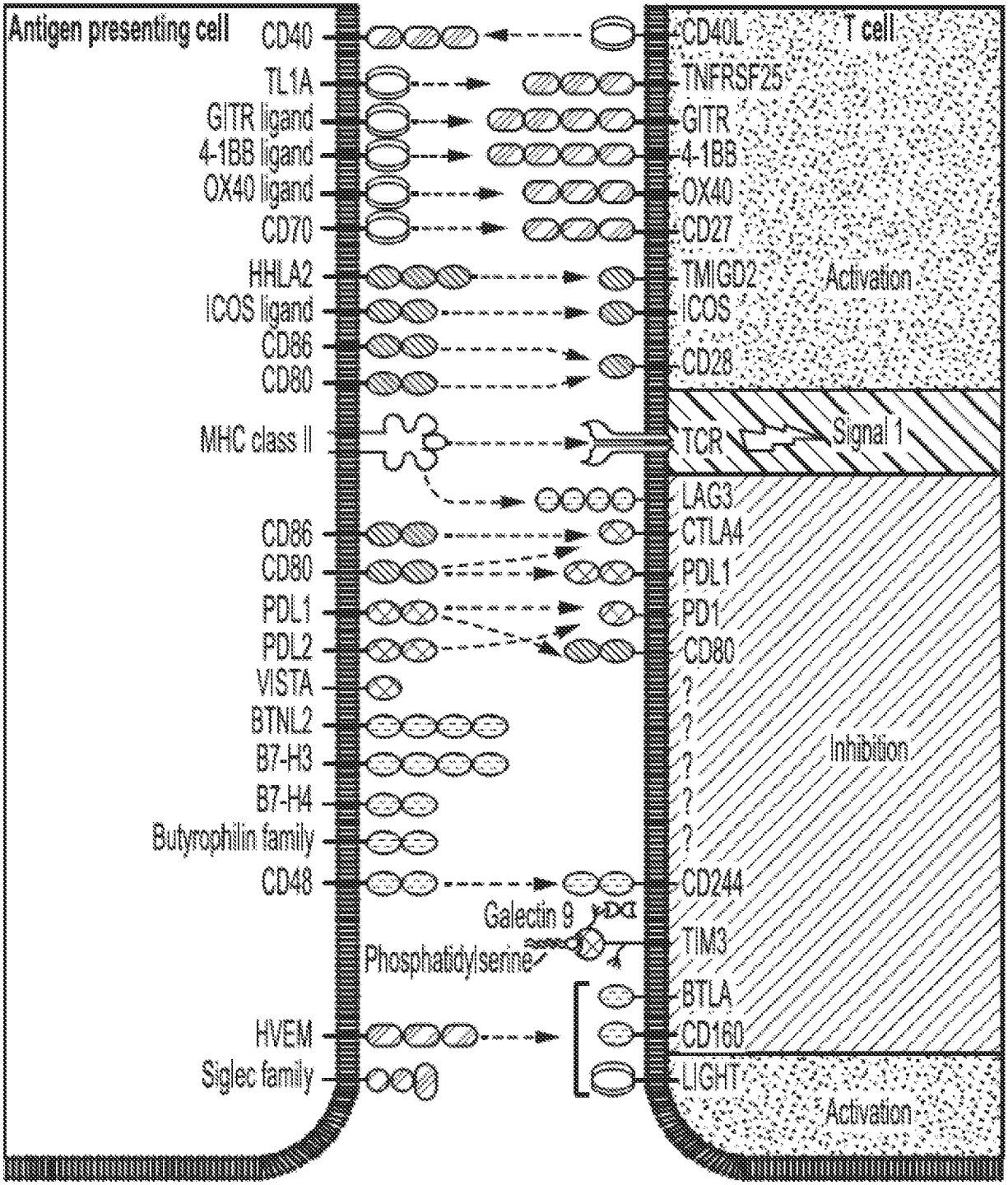

Antigen presenting cell
CD40
TL1A
GITR ligand
4-1BB ligand
OX40 ligand
CD70
HHLA2
ICOS ligand
CD86
CD80
MHC class II
CD86
CD80
PDL1
PDL2
VISTA
BTNL2
B7-H3
B7-H4
Butyrophilin family
CD48
HVEM
Siglec family
T cell
CD40L
TNFRSF25
GITR
4-1BB
OX40
CD27
TMIGD2
ICOS
CD28
Activation
TCR
Signal 1
LAG3
CTLA4
PDL1
PD1
CD80
?
?
?
?
?
?
Inhibition
CD244
Galectin 9
Phosphatidylserine
TIM3
BTLA
CD160
LIGHT
Activation

FIG. 3A

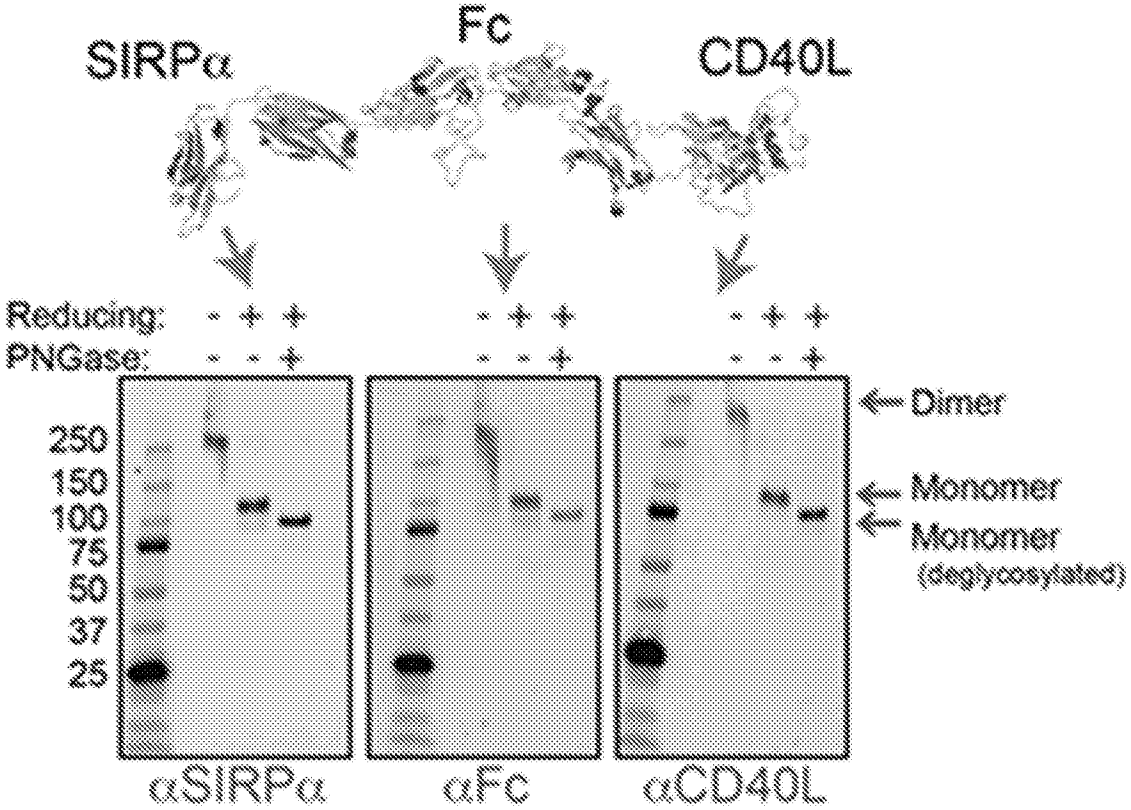

SIRPα
Fc
CD40L
Reducing: - + + - + + - + +
PNGase: - - + - - + - - +
250
150
100
75
50
37
25
Dimer
Monomer
Monomer
(deglycosylated)
αSIRPα
αFc
αCD40L

FIG. 3B

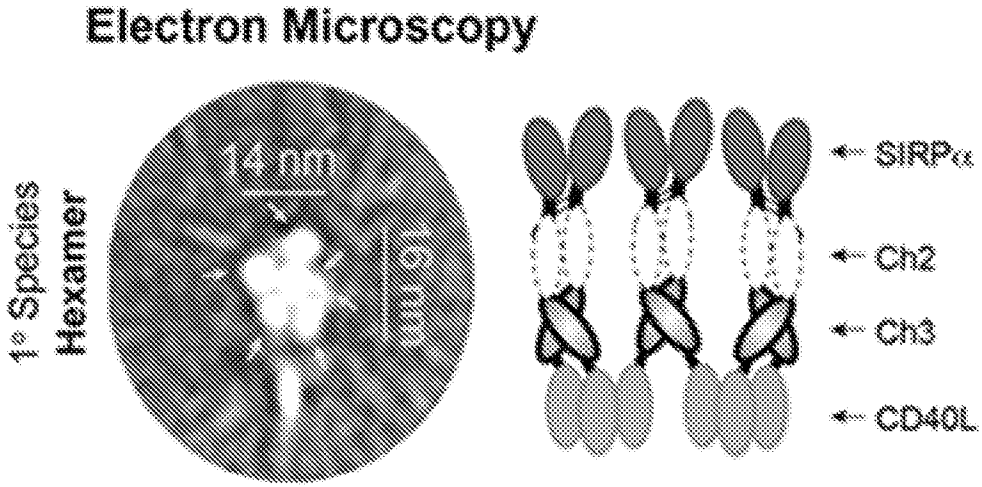

Electron Microscopy
1° Species
Hexamer
14 nm
19 nm
SIRPα
Ch2
Ch3
CD40L

FIG. 3C

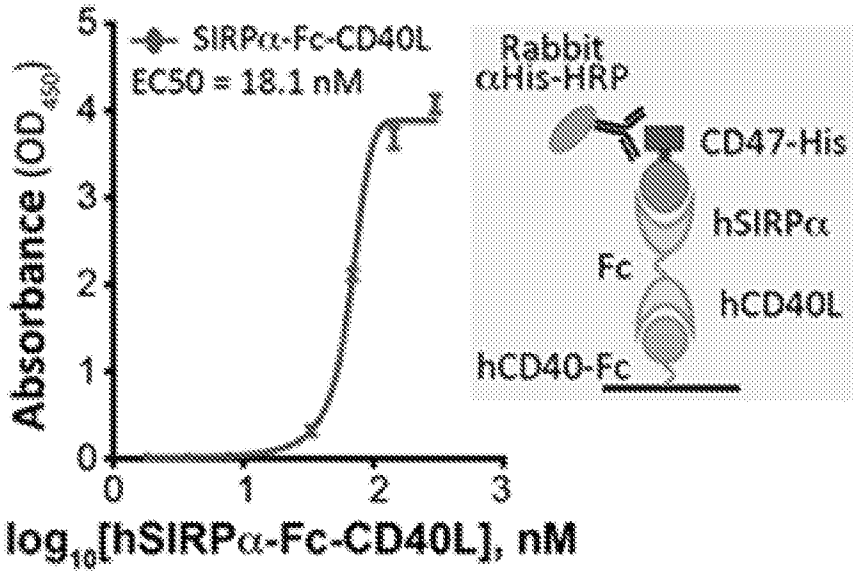

SIRPα-Fc-CD40L
EC50 = 18.1 nM
Absorbance (OD450)
log10[hSIRPα-Fc-CD40L], nM
Rabbit αHis-HRP
CD47-His
hSIRPα
Fc
hCD40L
hCD40-Fc

FIG. 3D

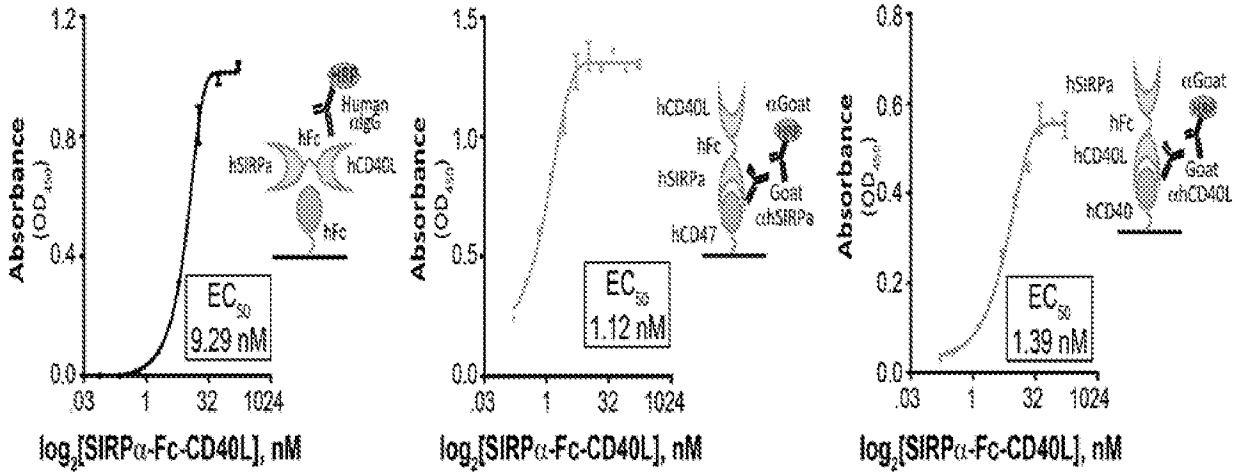

Absorbance (OD450)
log2[SIRPα-Fc-CD40L], nM
EC50 9.29 nM
Human αIgG
hFc
hSIRPa
hCD40L
EC50 1.12 nM
hCD40L
αGoat
hSIRPa
Goat αhSIRPa
hCD47
EC50 1.39 nM
hSIRPa
αGoat
hFc
hCD40L
Goat αhCD40L
hCD40

FIG. 3E

		Sample	Ka (on-rate; 1/Ms)	Kd (off-rate; 1/s)	KD (binding; M)
SPR Binding	CD47	SIRPα-Fc	1.31 E+6	2.28 E-2	17.6 nM
		SIRPα-Fc-CD40L	2.92 E+5	1.84 E-4	.628 nM
	CD40	Fc-CD40L	3.04 E+5	3.51 E-4	1.15 nM
		SIRPα-Fc-CD40L	8.18 E+4	4.57 E-4	4.74 nM
	FcγR1A	IgG1	1.84 E+4	1.56 E-4	8.42 nM
		SIRPα-Fc-CD40L	ND	ND	ND
	FcRn	IgG1	1.78 E+5	4.67 E-3	2.62 nM
		SIRPα-Fc-CD40L	1.45 E+5	3.38 E-3	2.33 nM

FIG. 3F

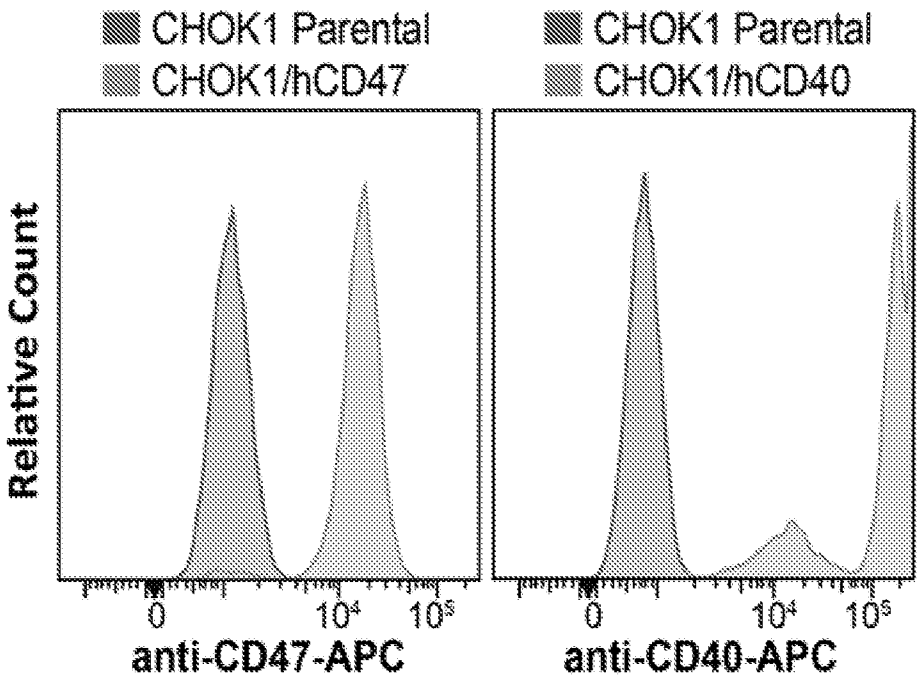


FIG. 3G

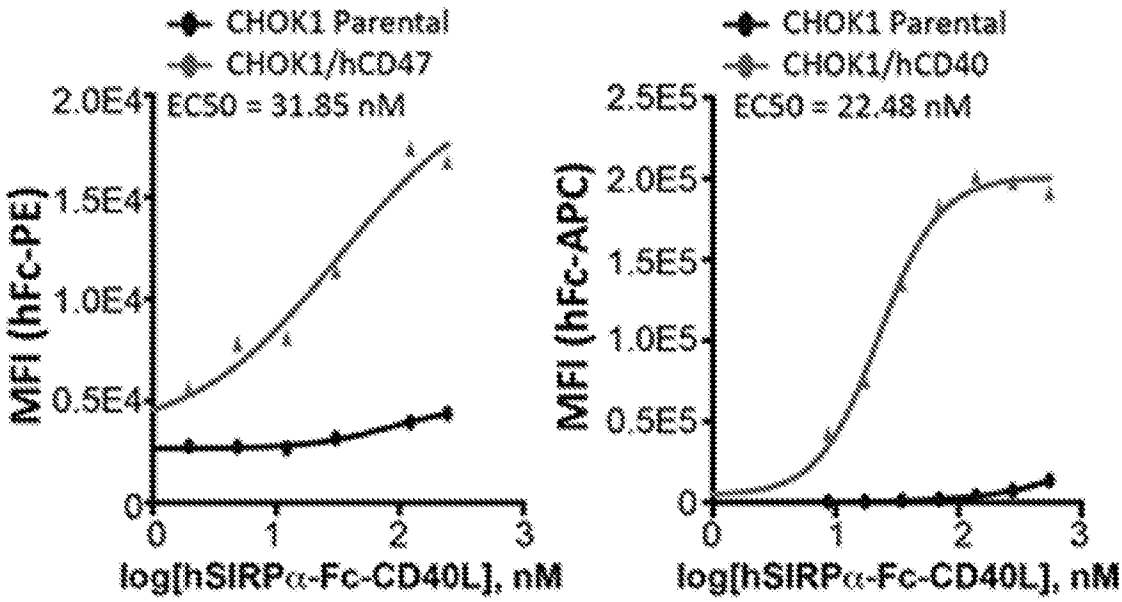

CHOK1 Parental
CHOK1/hCD47
EC50 = 31.85 nM
MFI (hFc-PE)
2.0E4
1.5E4
1.0E4
0.5E4
0
0
1
2
3
log[hSIRPα-Fc-CD40L], nM
CHOK1 Parental
CHOK1/hCD40
EC50 = 22.48 nM
MFI (hFc-APC)
2.5E5
2.0E5
1.5E5
1.0E5
0.5E5
0
0
1
2
3
log[hSIRPα-Fc-CD40L], nM

FIG. 3H

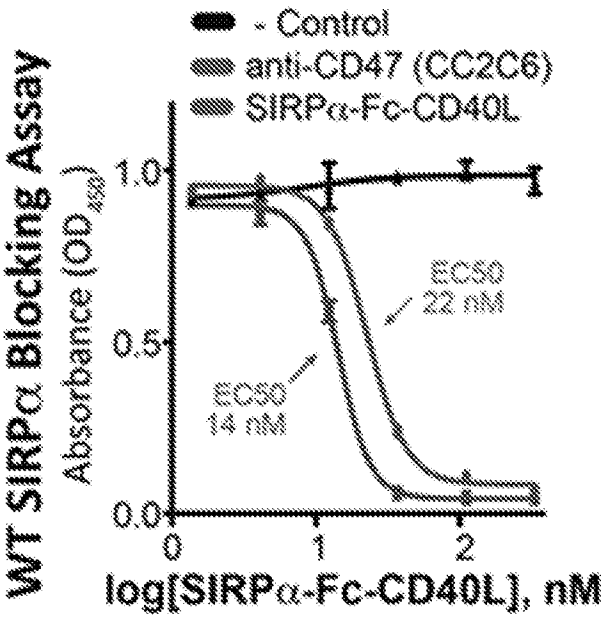

Control
anti-CD47 (CC2C6)
SIRPα-Fc-CD40L
WT SIRPα Blocking Assay
Absorbance (OD450)
1.0
0.5
0.0
EC50
22 nM
EC50
14 nM
0
1
2
log[SIRPα-Fc-CD40L], nM

FIG. 4A

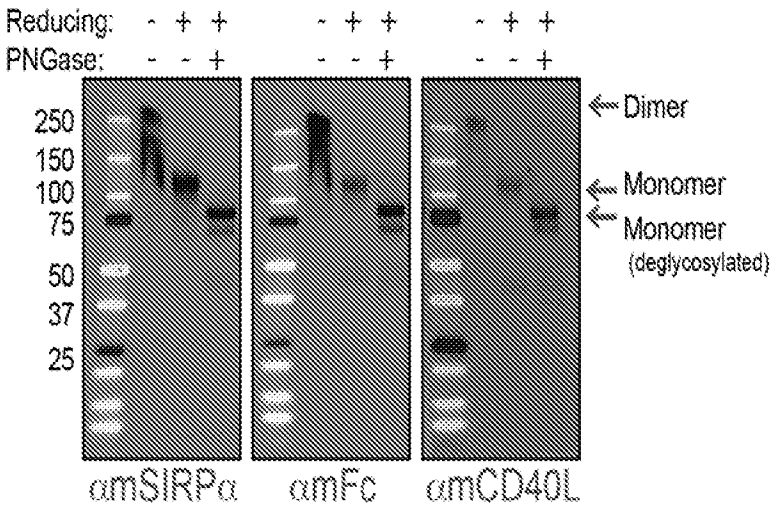

Reducing: - + + - + + - + +
PNGase: - - + - - + - - +
250
150
100
75
50
37
25
Dimer
Monomer
Monomer
(deglycosylated)
αmSIRPα
αmFc
αmCD40L

FIG. 4B

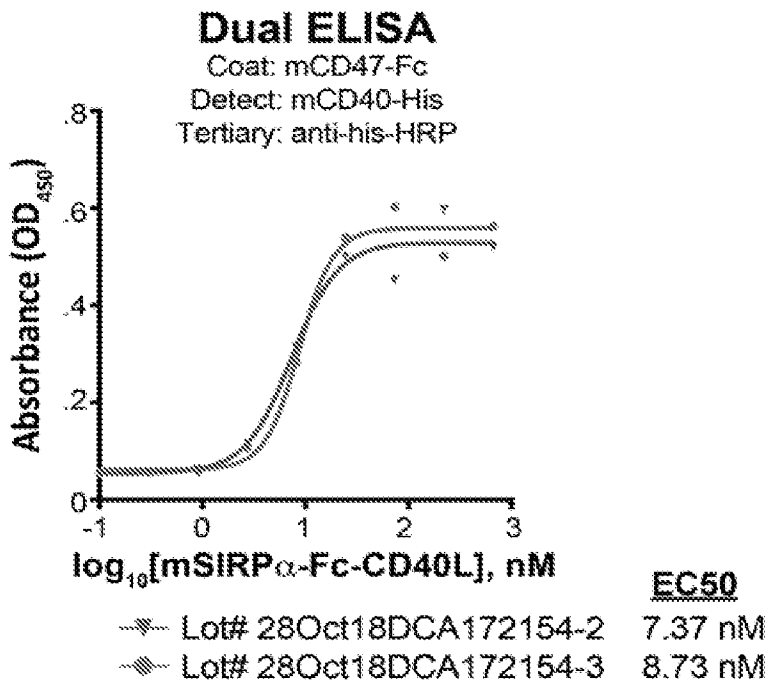

Dual ELISA
Coat: mCD47-Fc
Detect: mCD40-His
Tertiary: anti-his-HRP
Absorbance (OD450)
.8
.6
.4
.2
0
-1
0
1
2
3
log10[mSIRPα-Fc-CD40L], nM
EC50
Lot# 28Oct18DCA172154-2 7.37 nM
Lot# 28Oct18DCA172154-3 8.73 nM

FIG. 5A

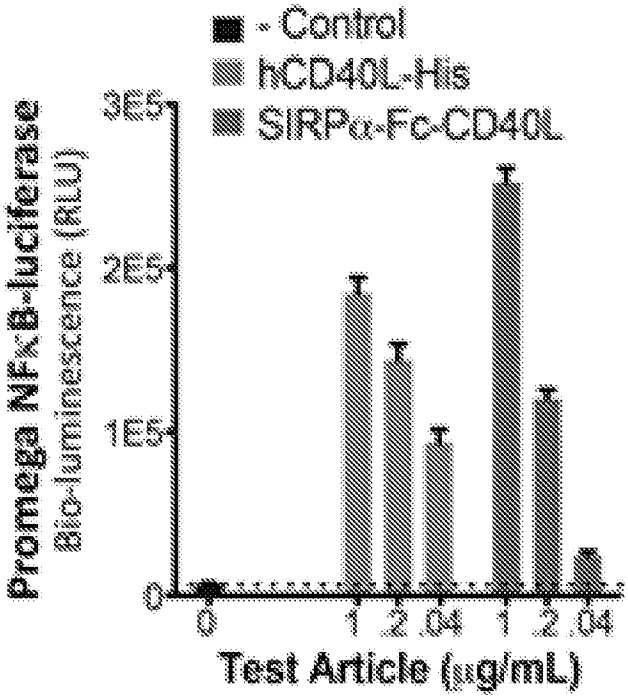

- Control
hCD40L-His
SIRPα-Fc-CD40L
Promega NFκB-luciferase
Bio-luminescence (RLU)
3E5
2E5
1E5
0
0
1
2
.04
1
2
.04
Test Article (μg/mL)

FIG. 5B

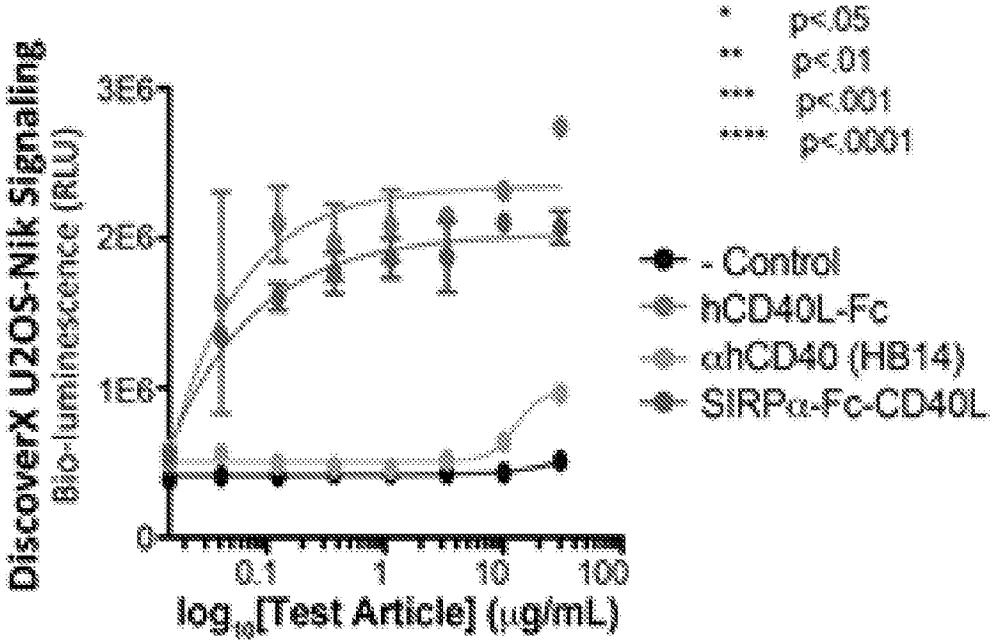

* p<.05
** p<.01
*** p<.001
**** p<.0001
DiscoverX U2OS-Nik Signaling
Bio-luminescence (RLU)
3E6
2E6
1E6
0
0.1
1
10
100
log10[Test Article] (μg/mL)
- Control
hCD40L-Fc
αhCD40 (HB14)
SIRPα-Fc-CD40L

FIG. 5C

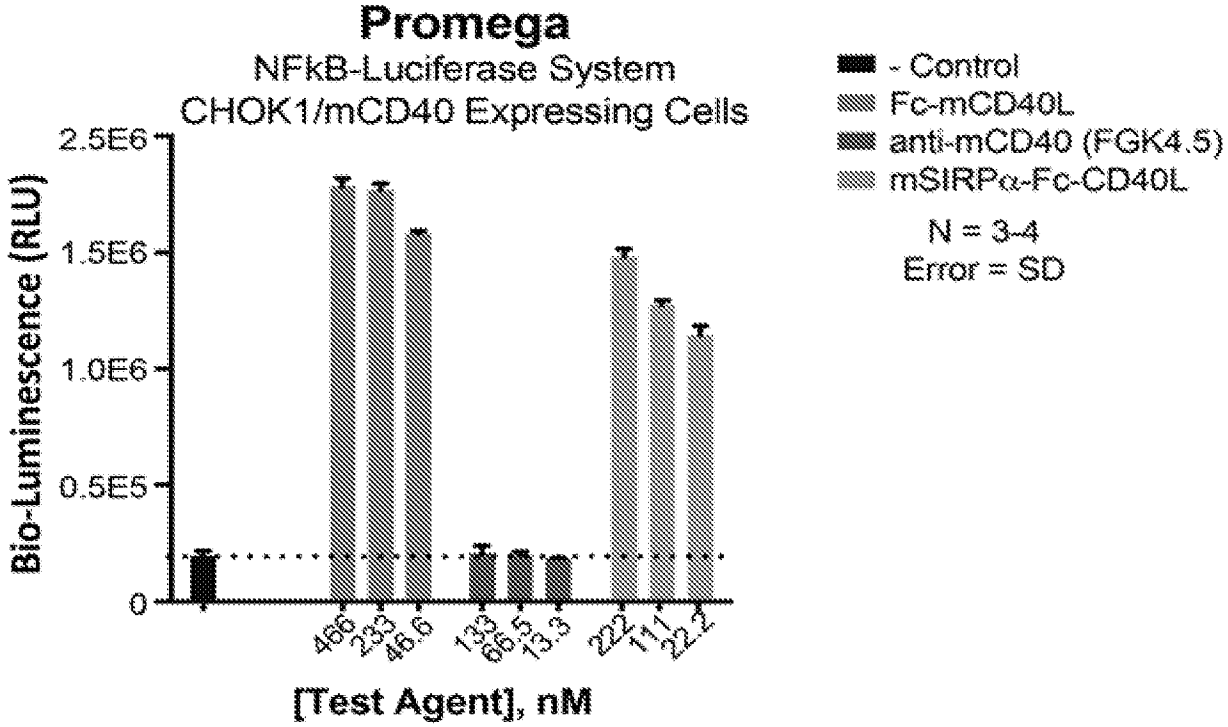

Promega
NFkB-Luciferase System
CHOK1/mCD40 Expressing Cells
Bio-Luminescence (RLU)
2.5E6
1.5E6
1.0E6
0.5E5
0
466
233
46.6
133
66.5
13.3
222
111
22.2
[Test Agent], nM
- Control
Fc-mCD40L
anti-mCD40 (FGK4.5)
mSIRPα-Fc-CD40L
N = 3-4
Error = SD

FIG. 6A

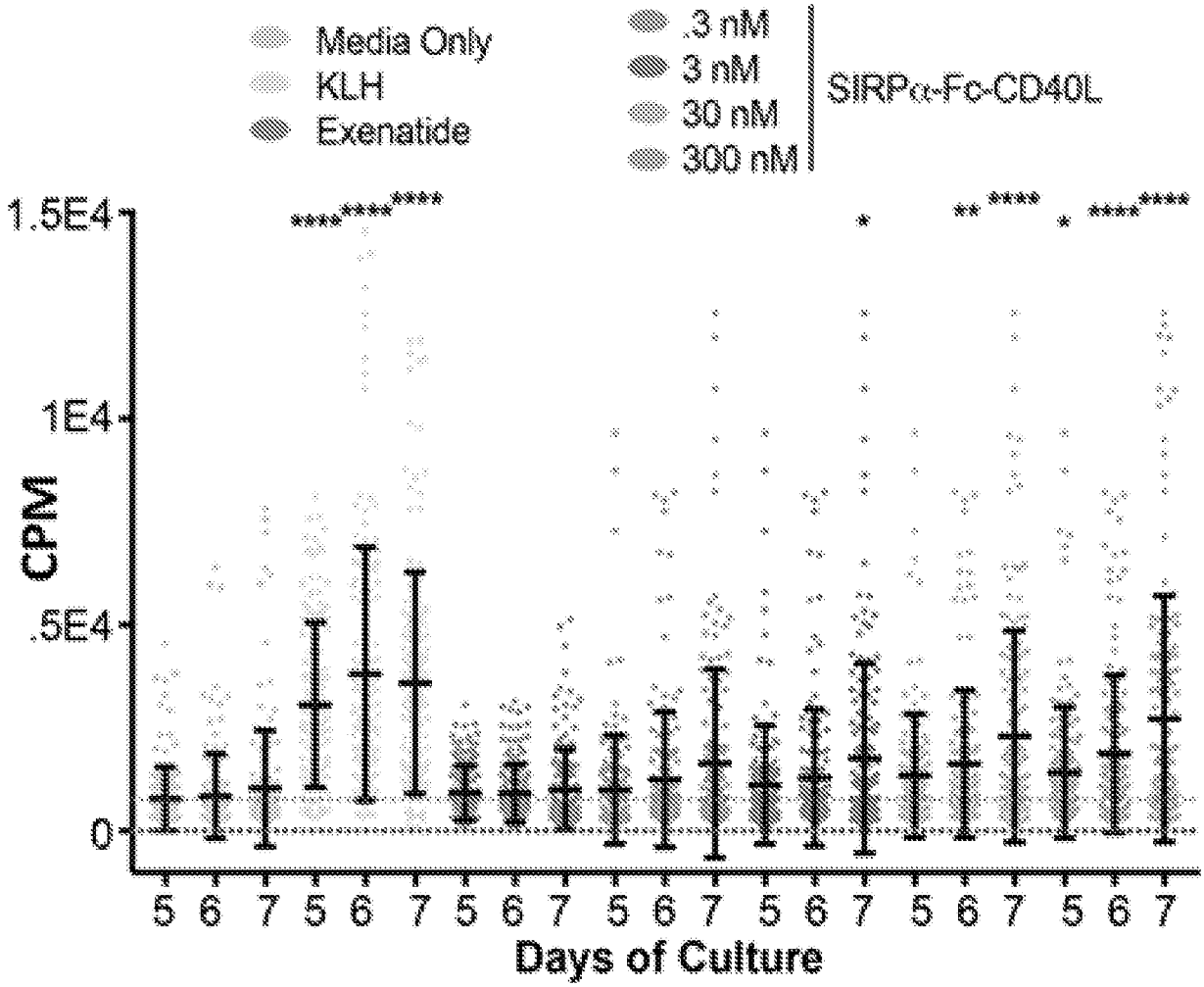

Media Only
KLH
Exenatide
.3 nM
3 nM
30 nM
300 nM
SIRPα-Fc-CD40L
1.5E4
1E4
.5E4
0
CPM
****
****
****
*
**
****
*
****
****
5 6 7 5 6 7 5 6 7 5 6 7 5 6 7 5 6 7 5 6 7
Days of Culture

FIG. 6B

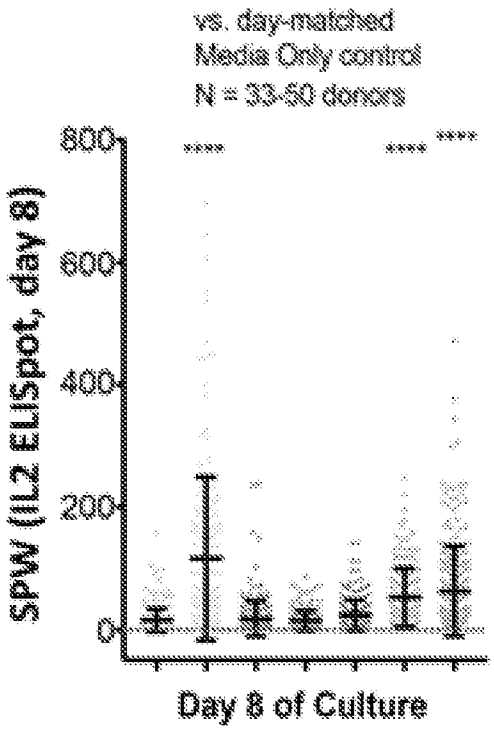


FIG. 7A

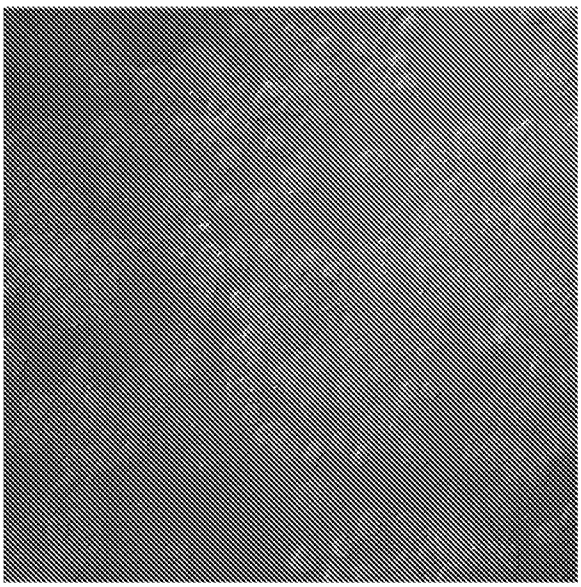


FIG. 7B

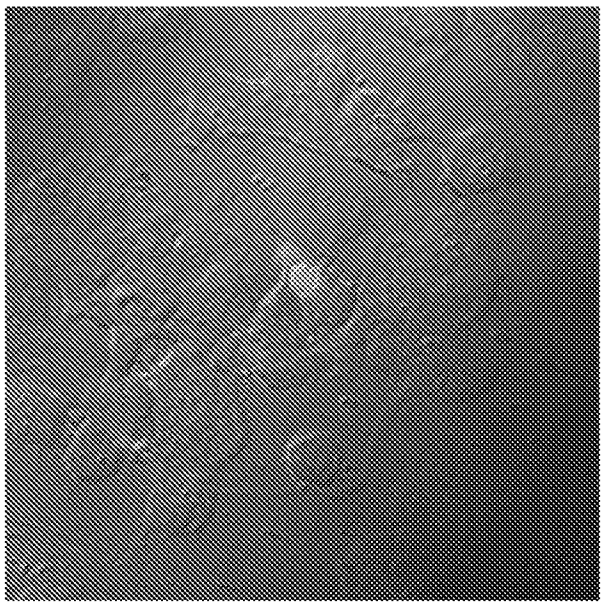


FIG. 7C



FIG. 7D

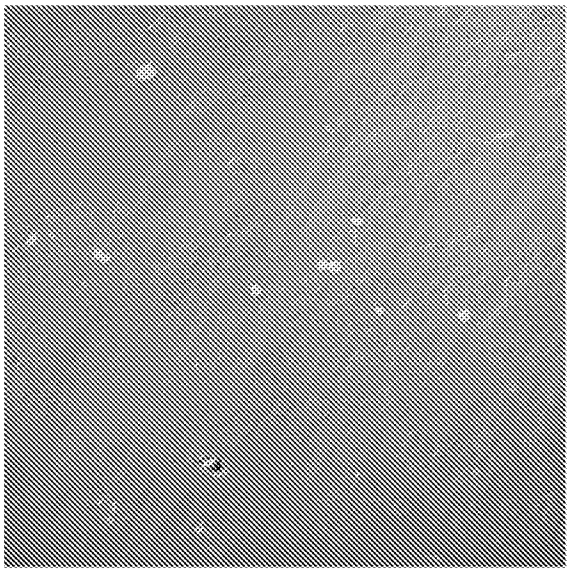


FIG. 7E

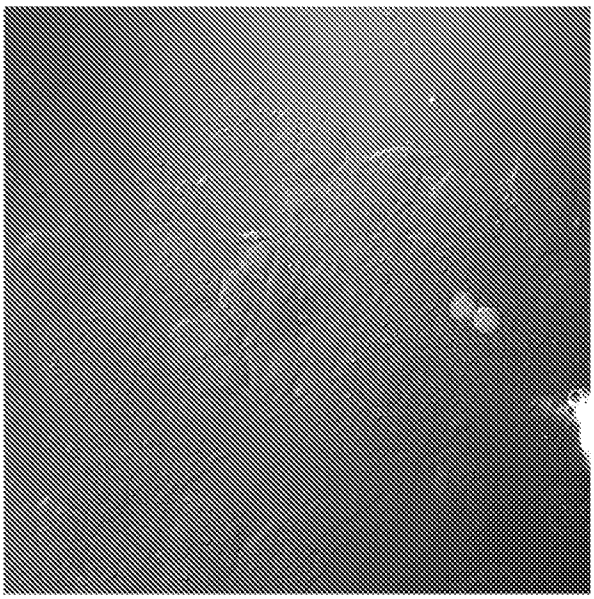


FIG. 7F

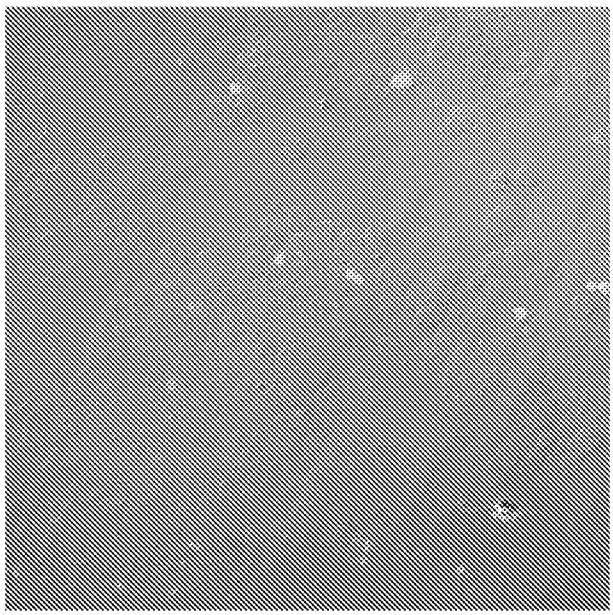


FIG. 7G

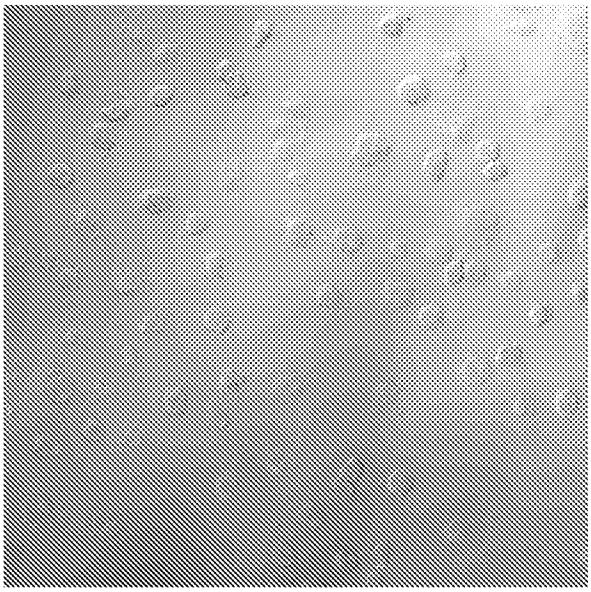


FIG. 7H

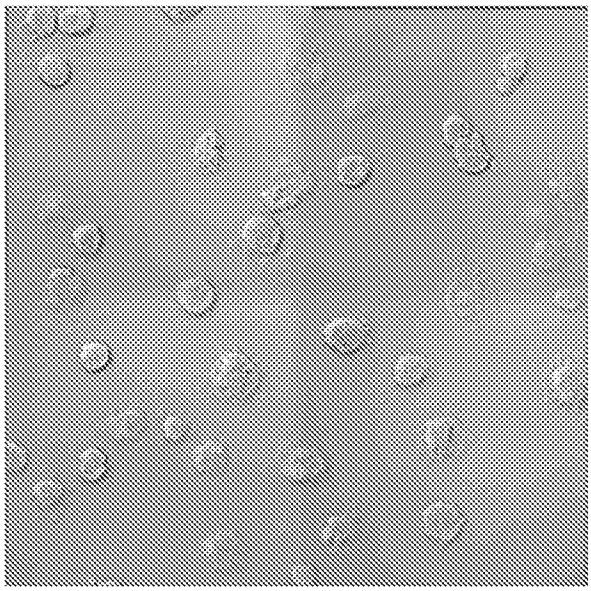


FIG. 8A

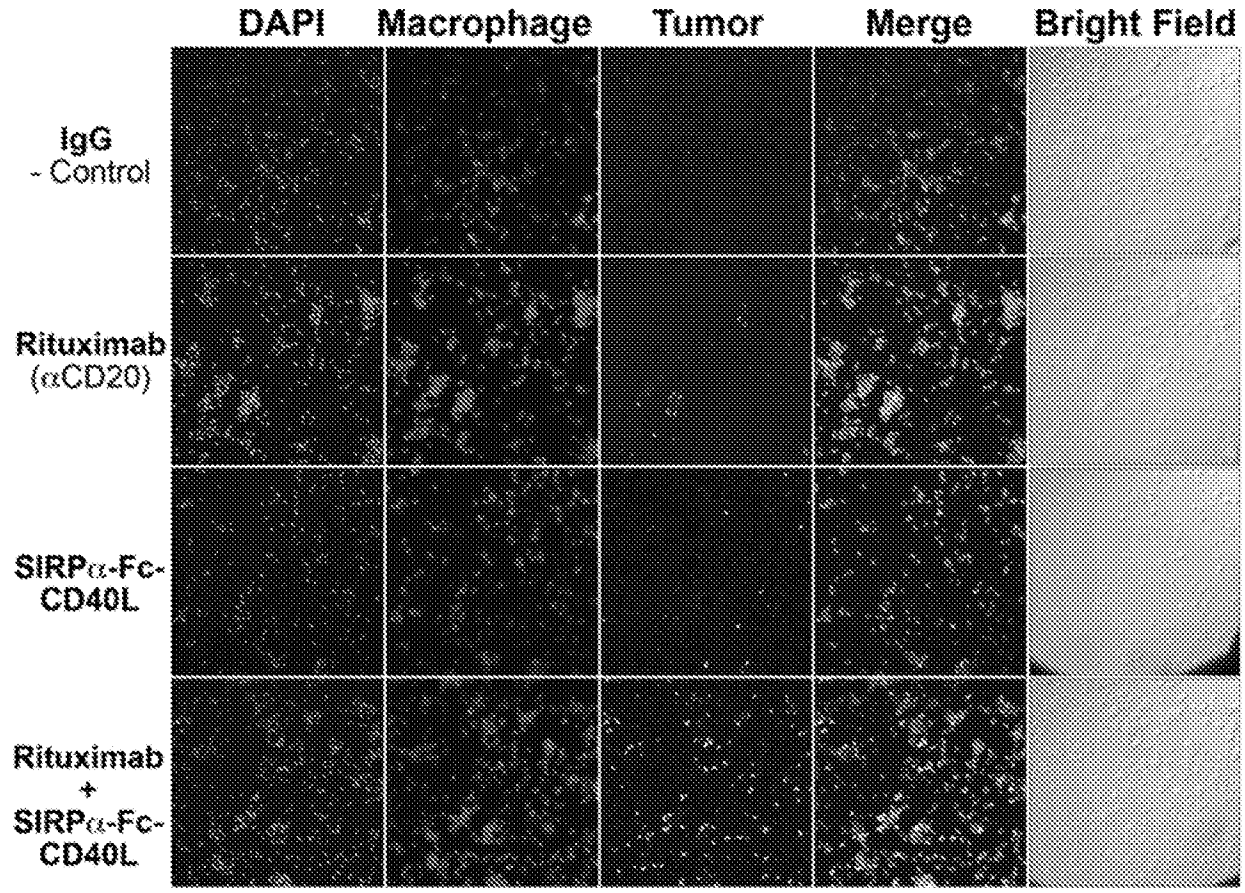


FIG. 8B

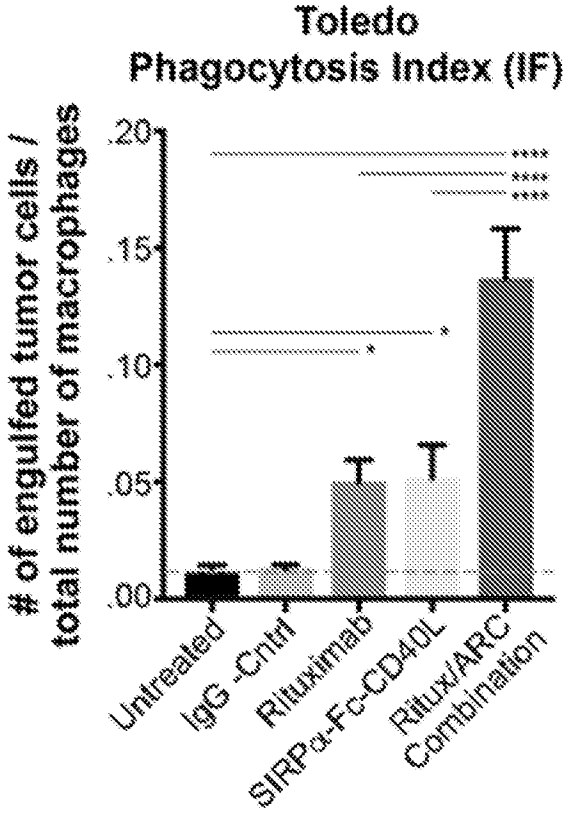

Toledo
Phagocytosis Index (IF)
# of engulfed tumor cells / total number of macrophages
.20
.15
.10
.05
.00
Untreated
IgG -Cntrl
Rituximab
SIRPα-Fc-CD40L
Ritux/ARC Combination

FIG 8C

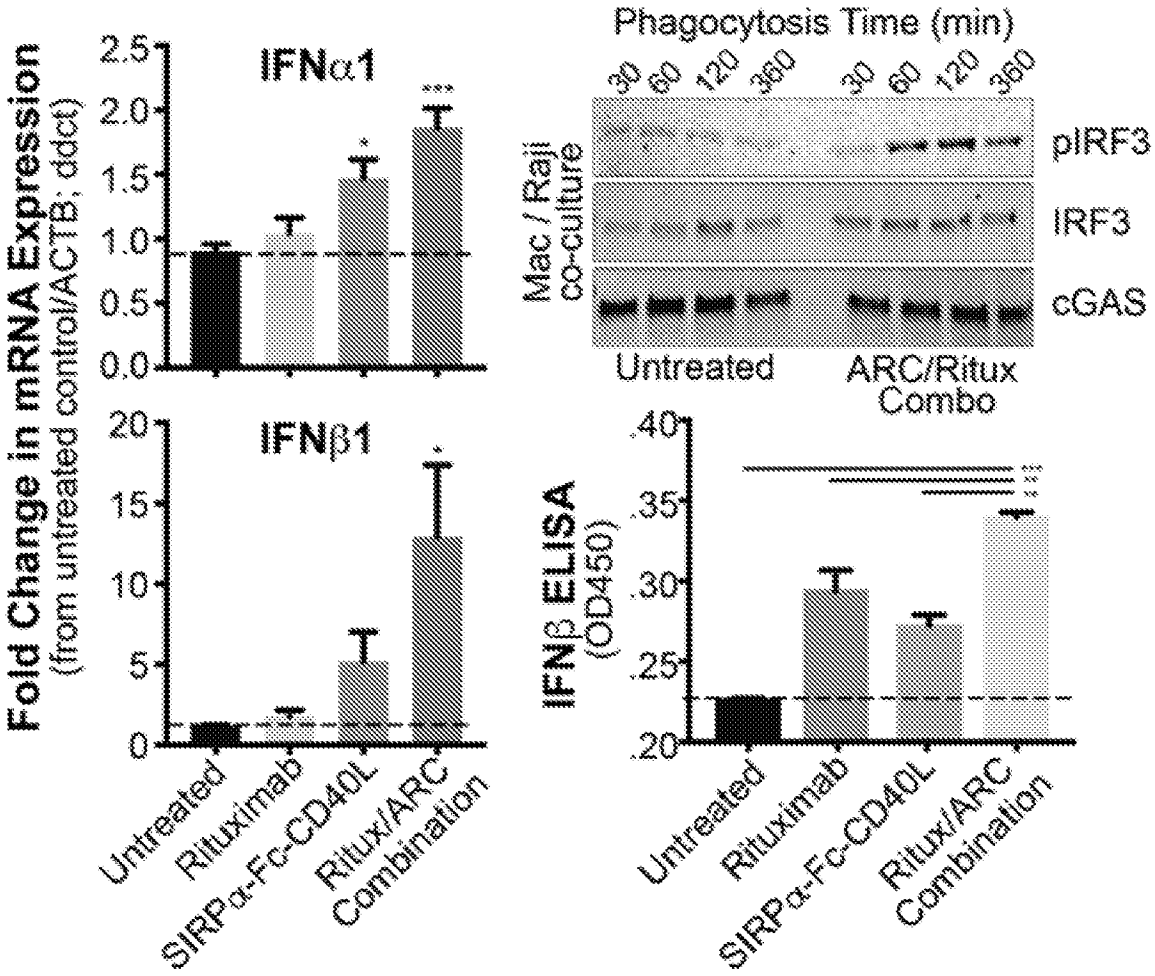

Fold Change in mRNA Expression
(from untreated control/ACTB; ddct)
IFNα1
IFNβ1
2.5
2.0
1.5
1.0
0.5
0.0
20
15
10
5
0
Untreated
Rituximab
SIRPα-Fc-CD40L
Ritux/ARC Combination
Phagocytosis Time (min)
30 60 120 360 30 60 120 360
Mac / Raji co-culture
pIRF3
IRF3
cGAS
Untreated
ARC/Ritux Combo
IFNβ ELISA
(OD450)
.40
.35
.30
.25
.20
Untreated
Rituximab
SIRPα-Fc-CD40L
Ritux/ARC Combination

FIG. 8D

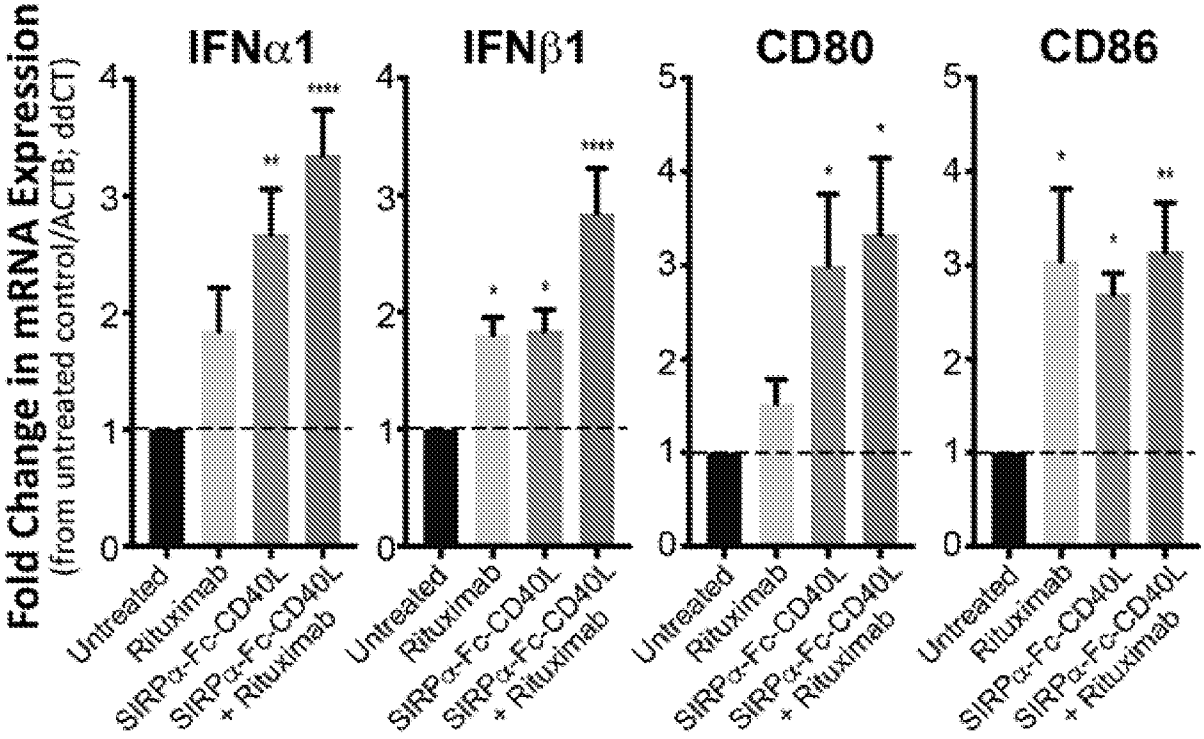

IFNα1
IFNβ1
CD80
CD86
Fold Change in mRNA Expression
(from untreated control/ACTB; ddCT)
Untreated
Rituximab
SIRPα-Fc-CD40L
SIRPα-Fc-CD40L + Rituximab

FIG. 8E

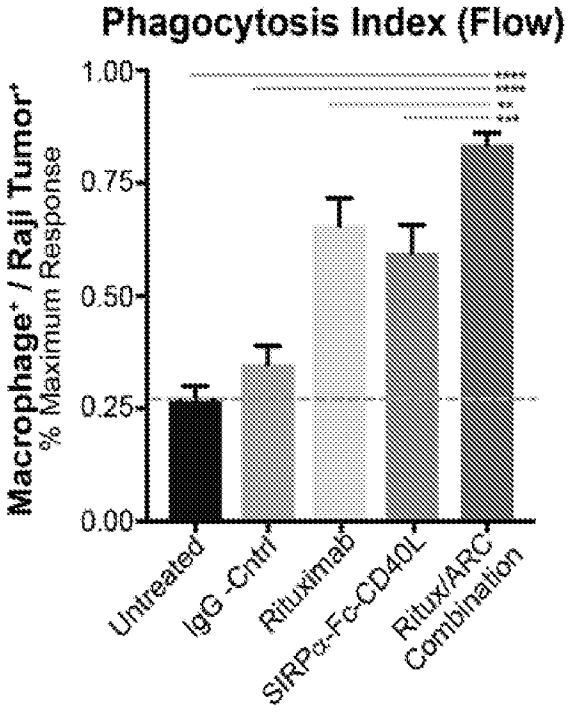

Phagocytosis Index (Flow)
Macrophage+ / Raji Tumor+
% Maximum Response
Untreated
IgG -Cntrl
Rituximab
SIRPα-Fc-CD40L
Ritux/ARC Combination

FIG 8F

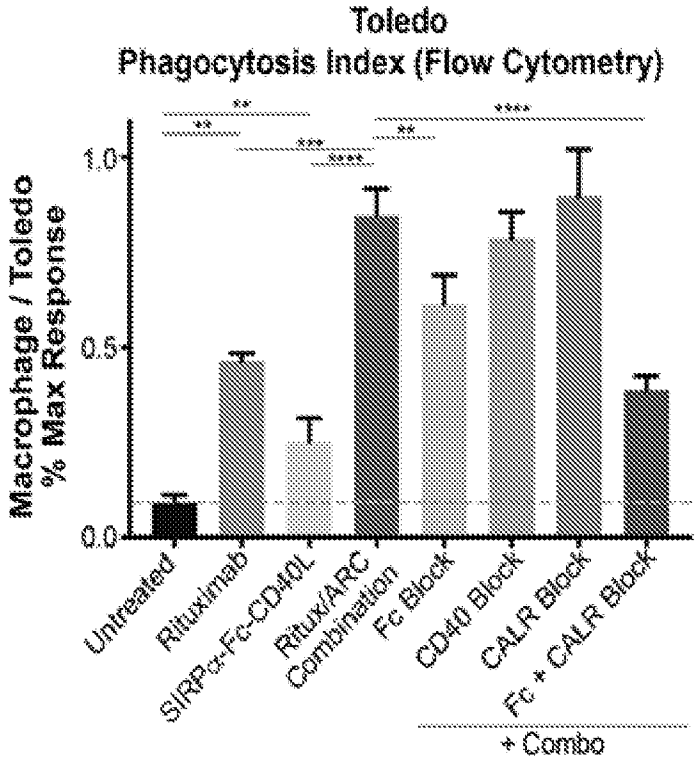

Toledo
Phagocytosis Index (Flow Cytometry)
Macrophage / Toledo
% Max Response
1.0
0.5
0.0
Untreated
Rituximab
SIRPα-Fc-CD40L
Ritux/ARC Combination
Fc Block
CD40 Block
CALR Block
Fc + CALR Block
+ Combo

FIG. 8G

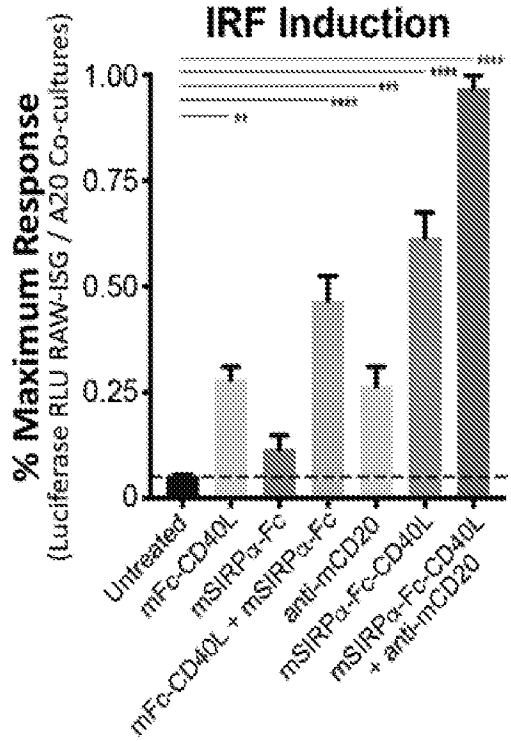

IRF Induction
% Maximum Response
(Luciferase RLU RAW-ISG / A20 Co-cultures)
1.00
0.75
0.50
0.25
0
Untreated
mFc-CD40L
mSIRPα-Fc
mFc-CD40L + mSIRPα-Fc
anti-mCD20
mSIRPα-Fc-CD40L
mSIRPα-Fc-CD40L + anti-mCD20

FIG. 8H

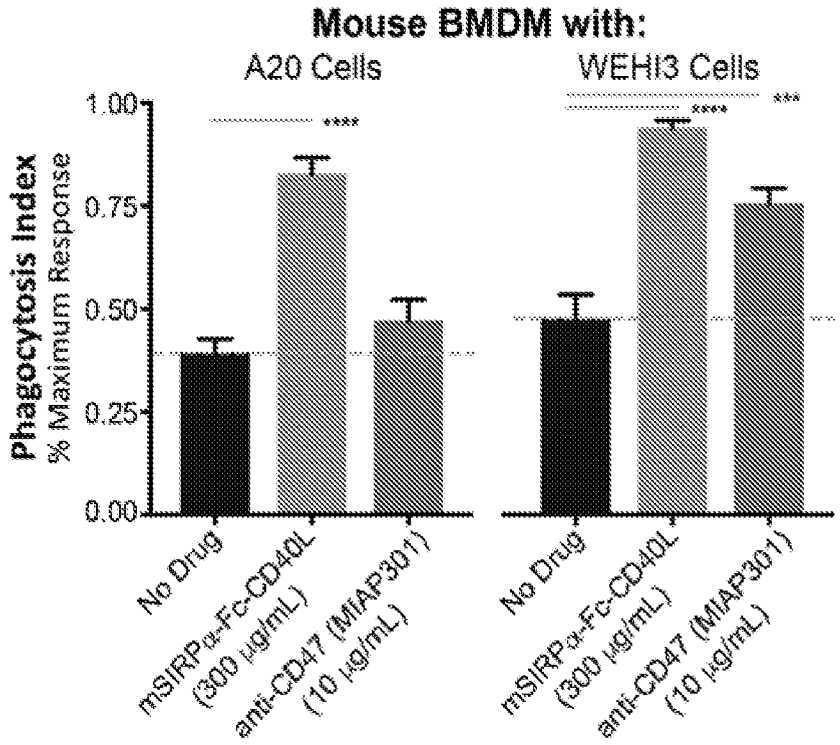

Mouse BMDM with:
A20 Cells
WEHI3 Cells
Phagocytosis Index
% Maximum Response
1.00
0.75
0.50
0.25
0.00
****
****
***
No Drug
mSIRPα-Fc-CD40L (300 μg/mL)
anti-CD47 (MIAP301) (10 μg/mL)
No Drug
mSIRPα-Fc-CD40L (300 μg/mL)
anti-CD47 (MIAP301) (10 μg/mL)

FIG. 9A

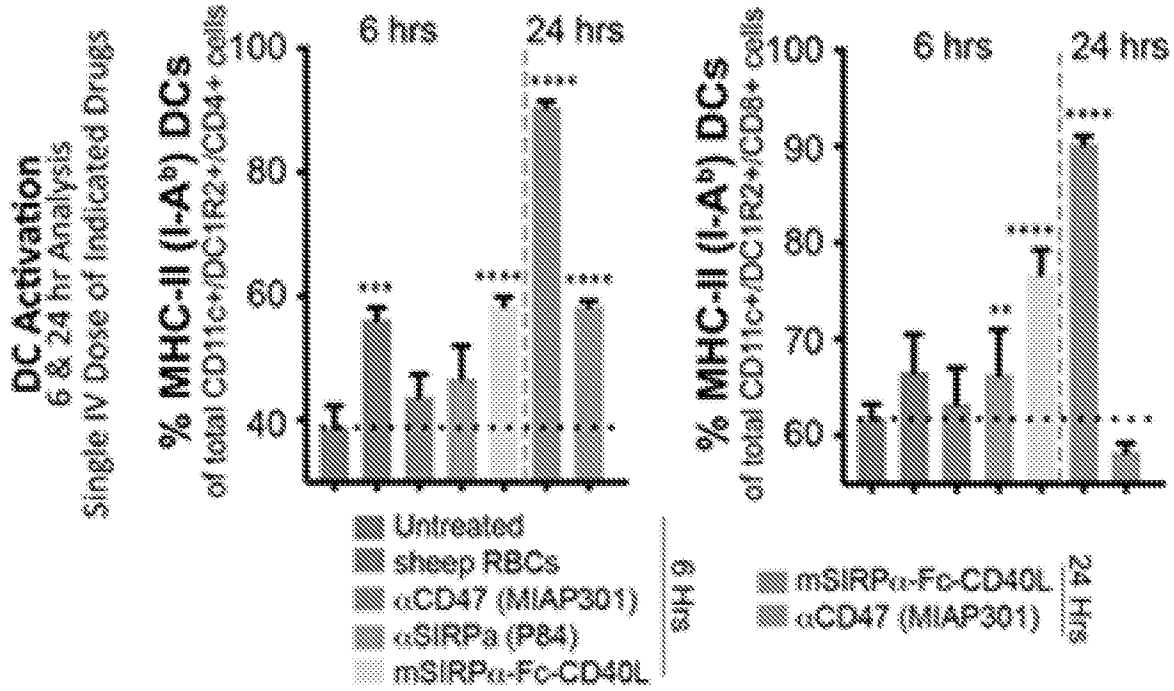

DC Activation
6 & 24 hr Analysis
Single IV Dose of Indicated Drugs
% MHC-II (I-Ab) DCs
of total CD11c+/DC1R2+/CD4+ cells
100
80
60
40
6 hrs
24 hrs
% MHC-II (I-Ab) DCs
of total CD11c+/DC1R2+/CD8+ cells
100
90
80
70
60
6 hrs
24 hrs
Untreated
sheep RBCs
αCD47 (MIAP301)
αSIRPa (P84)
mSIRPα-Fc-CD40L
6 Hrs
mSIRPα-Fc-CD40L
αCD47 (MIAP301)
24 Hrs

FIG. 9B

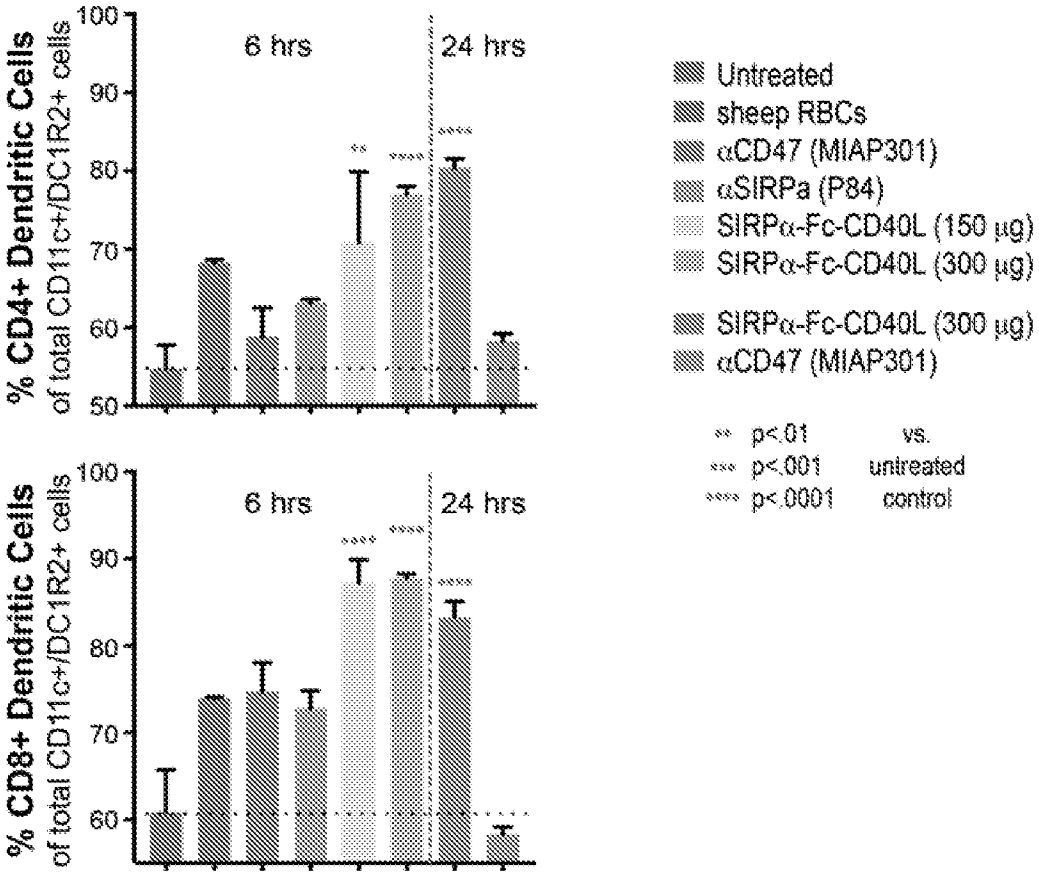


FIG. 10A

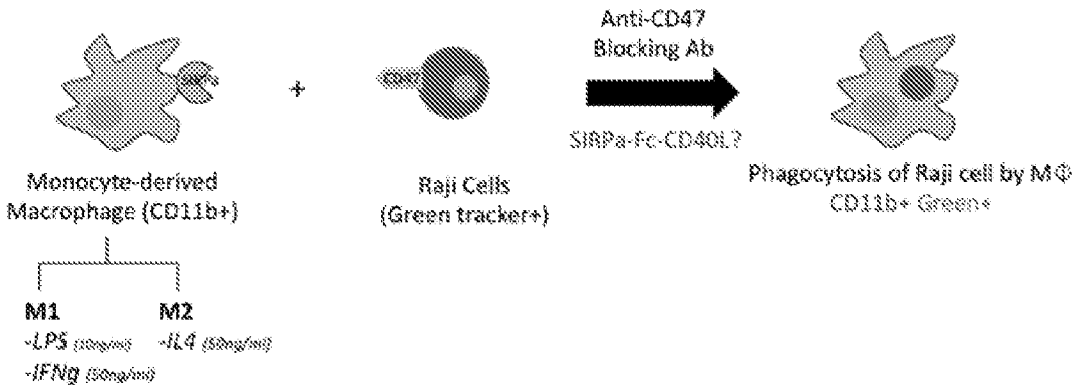


FIG. 10B

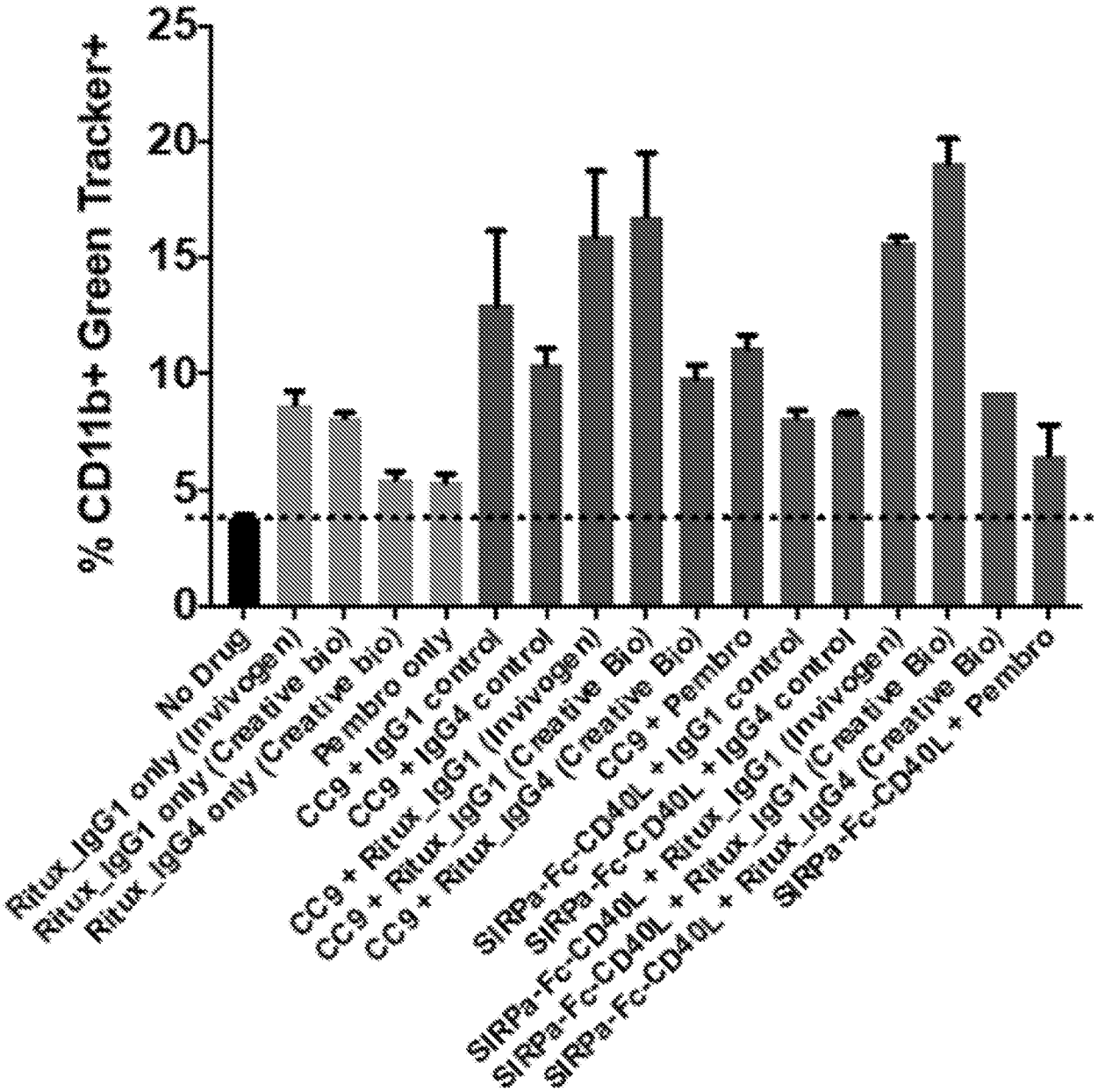

% CD11b+ Green Tracker+
25
20
15
10
5
0
No Drug
Ritux_IgG1 only (Invivogen)
Ritux_IgG1 only (Creative bio)
Ritux_IgG4 only (Creative bio)
Pembro only
CC9 + IgG1 control
CC9 + IgG4 control
CC9 + Ritux_IgG1 (Invivogen)
CC9 + Ritux_IgG1 (Creative Bio)
CC9 + Ritux_IgG4 (Creative Bio)
CC9 + Pembro
SIRPa-Fc-CD40L + IgG1 control
SIRPa-Fc-CD40L + IgG4 control
SIRPa-Fc-CD40L + Ritux_IgG1 (Invivogen)
SIRPa-Fc-CD40L + Ritux_IgG1 (Creative Bio)
SIRPa-Fc-CD40L + Ritux_IgG4 (Creative Bio)
SIRPa-Fc-CD40L + Pembro

FIG. 10C

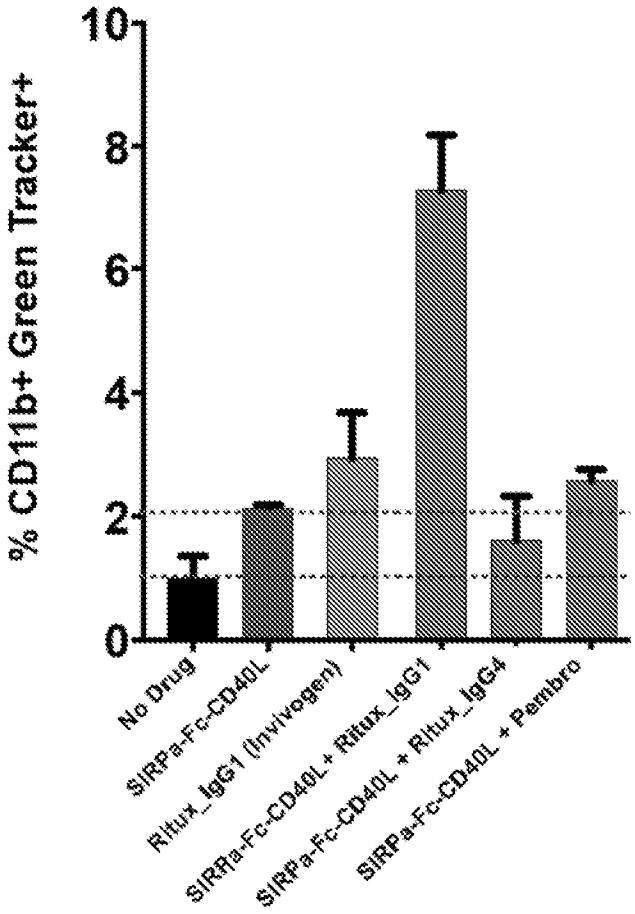

% CD11b+ Green Tracker+
10
8
6
4
2
0
No Drug
SIRPa-Fc-CD40L
Ritux_IgG1 (Invivogen)
SIRPa-Fc-CD40L + Ritux_IgG1
SIRPa-Fc-CD40L + Ritux_IgG4
SIRPa-Fc-CD40L + Pembro

FIG. 11

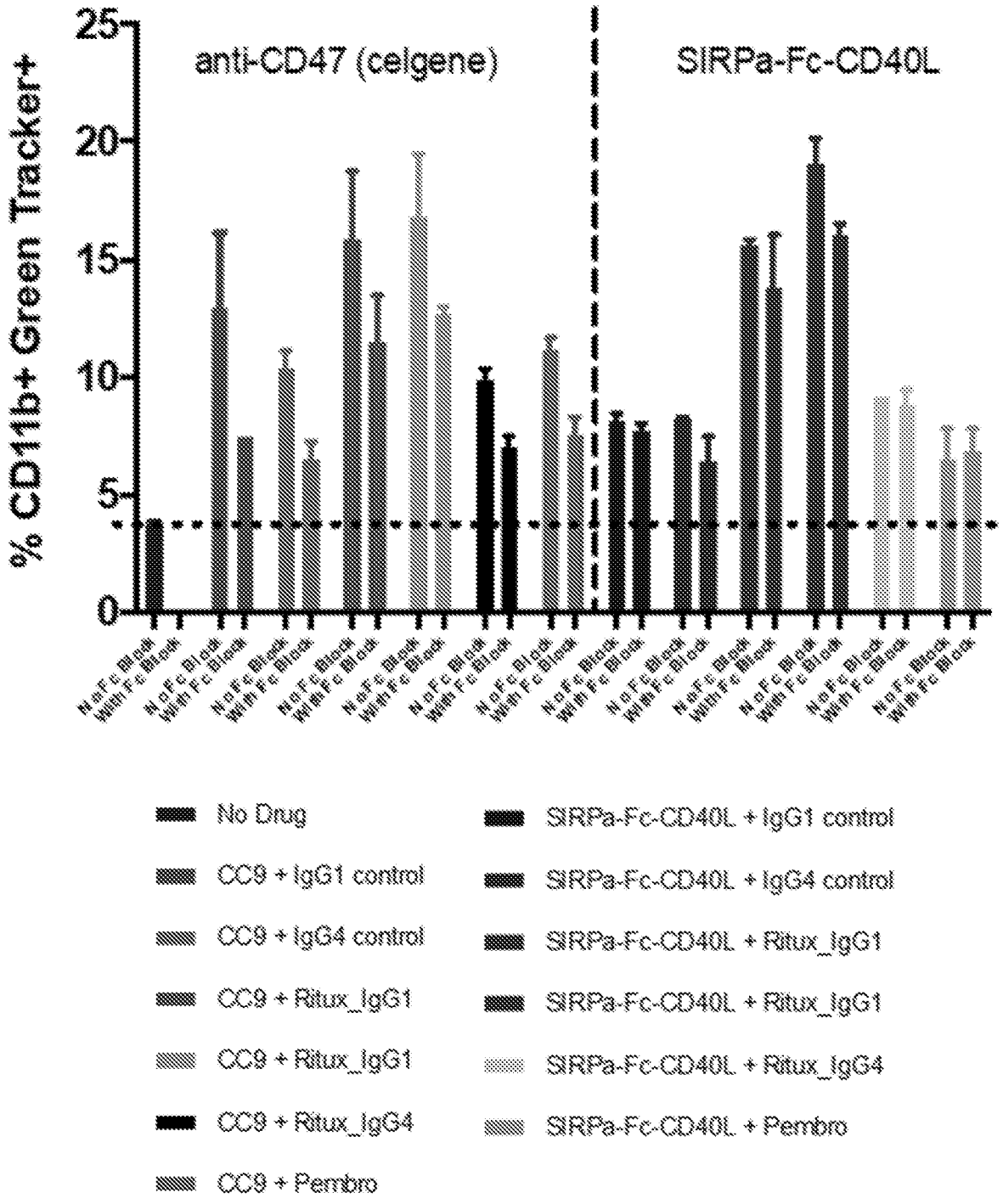
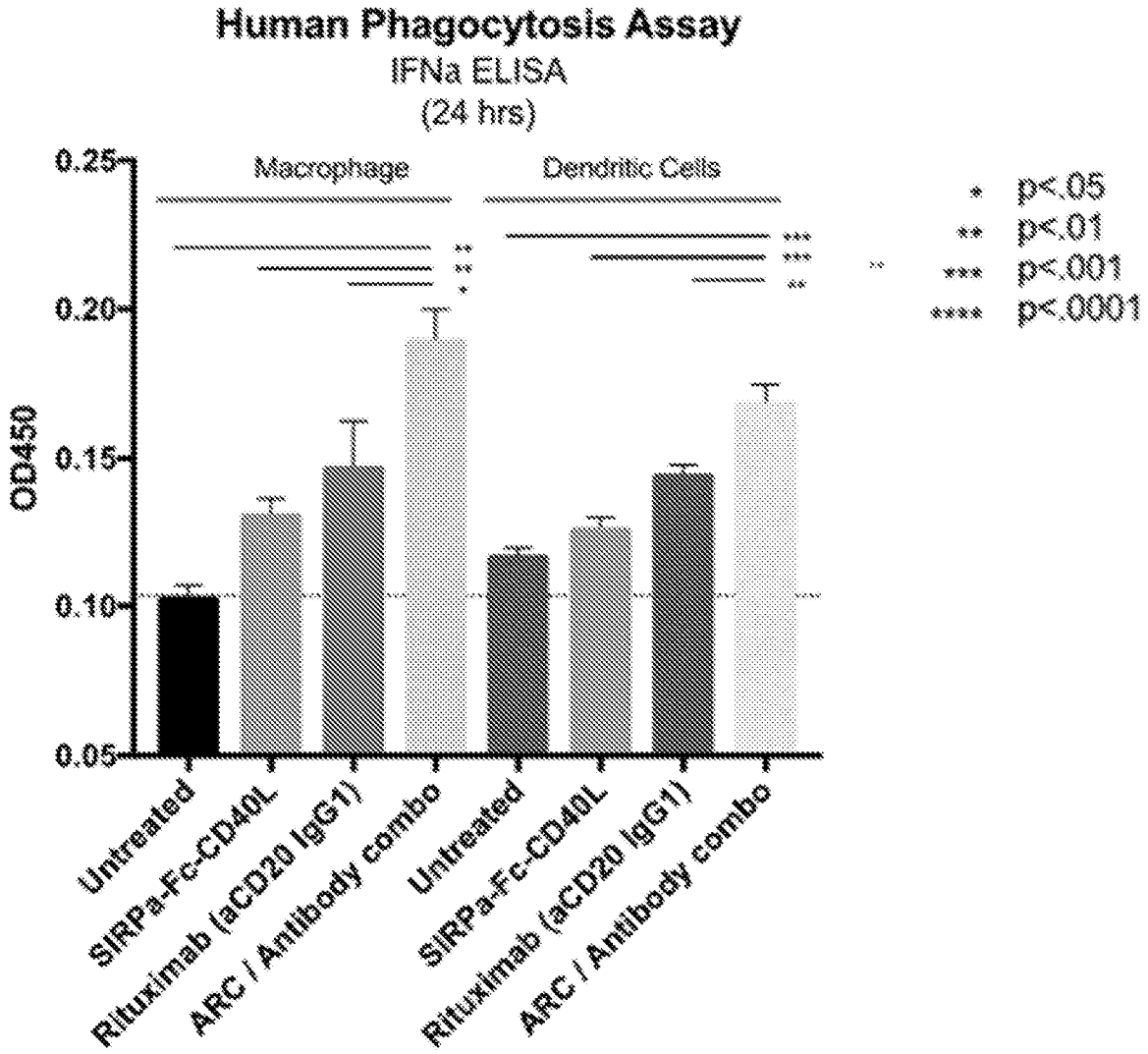

% CD11b+ Green Tracker+
25
20
15
10
5
0
anti-CD47 (celgene)
SIRPa-Fc-CD40L
No Fc Block
With Fc Block
No Drug
CC9 + IgG1 control
CC9 + IgG4 control
CC9 + Ritux_IgG1
CC9 + Ritux_IgG1
CC9 + Ritux_IgG4
CC9 + Pembro
SIRPa-Fc-CD40L + IgG1 control
SIRPa-Fc-CD40L + IgG4 control
SIRPa-Fc-CD40L + Ritux_IgG1
SIRPa-Fc-CD40L + Ritux_IgG1
SIRPa-Fc-CD40L + Ritux_IgG4
SIRPa-Fc-CD40L + Pembro

FIG. 12A



IFNa Multi-Subtype

IFNaA, IFNa2, IFNaD,
IFNaB2, IFNaC, IFNaG,
IFNaH, IFNaI, IFNaJ1,
IFNaK, IFNa1, IFNa4A,
IFNa4B, IFNaWA

FIG. 12B

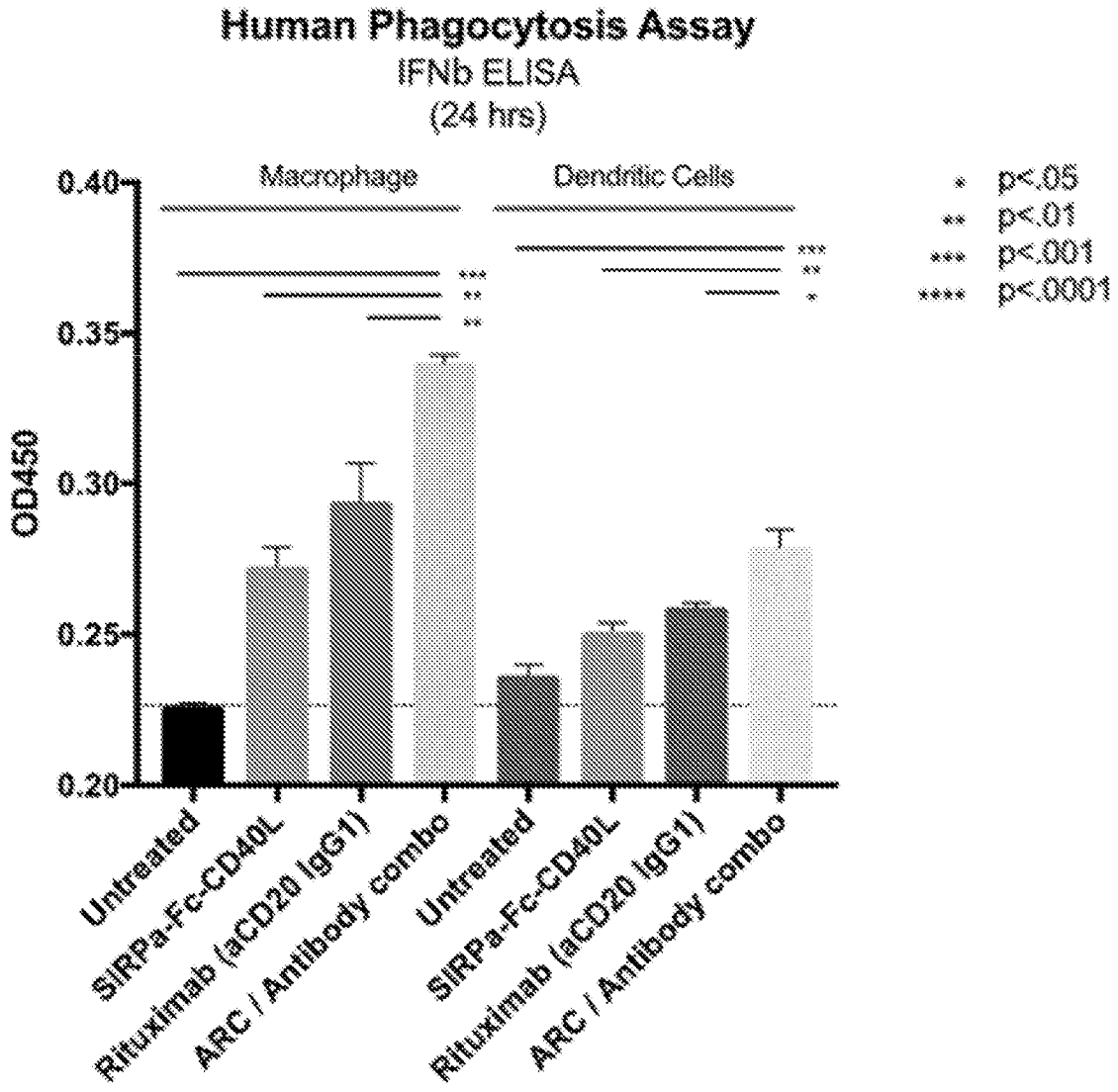

Human Phagocytosis Assay
IFNb ELISA
(24 hrs)
Macrophage
Dendritic Cells
* p<.05
** p<.01
*** p<.001
**** p<.0001
OD450
0.40
0.35
0.30
0.25
0.20
Untreated
SIRPa-Fc-CD40L
Rituximab (aCD20 IgG1)
ARC / Antibody combo
Untreated
SIRPa-Fc-CD40L
Rituximab (aCD20 IgG1)
ARC / Antibody combo

FIG. 13A

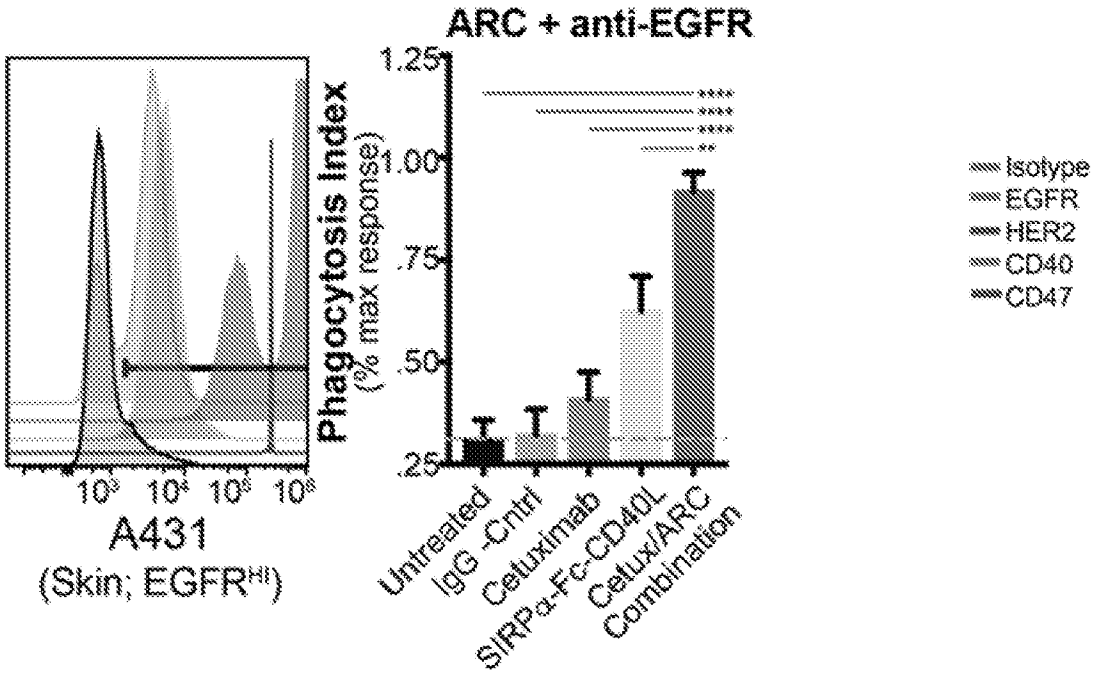


FIG. 13B

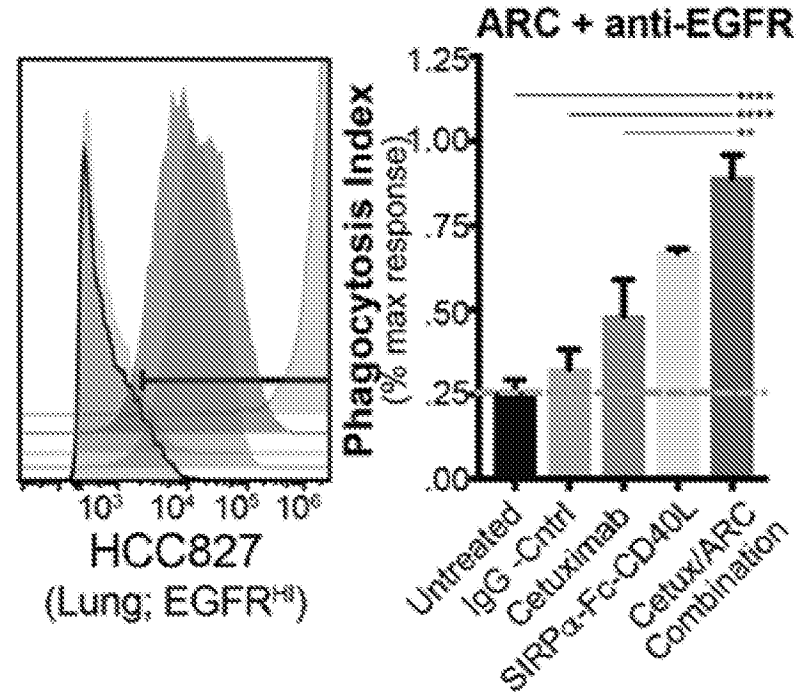


FIG. 13C

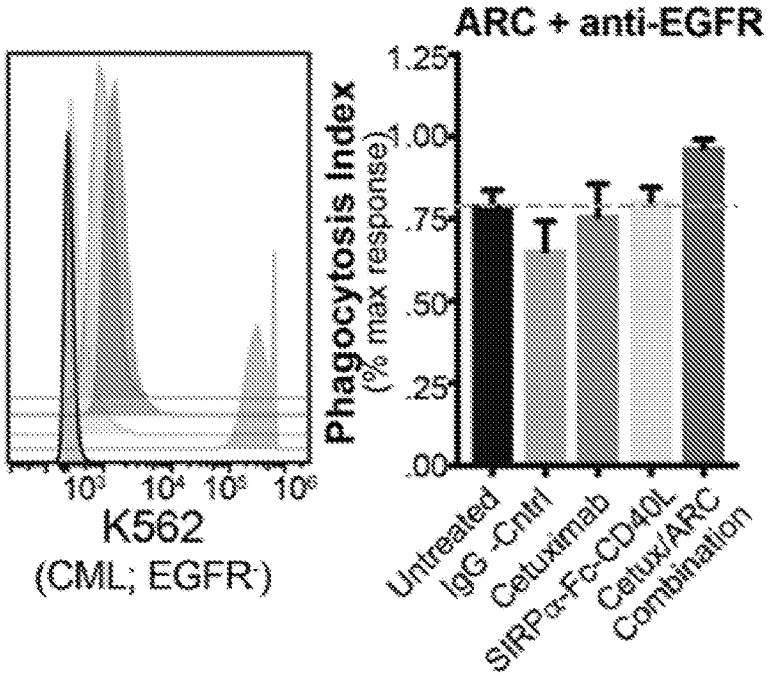

ARC + anti-EGFR
Phagocytosis Index
(% max response)
1.25
1.00
.75
.50
.25
.00
10^3 10^4 10^5 10^6
K562
(CML; EGFR-)
Untreated
IgG -Cntrl
Cetuximab
SIRPα-Fc-CD40L
Cetux/ARC
Combination

FIG. 14A

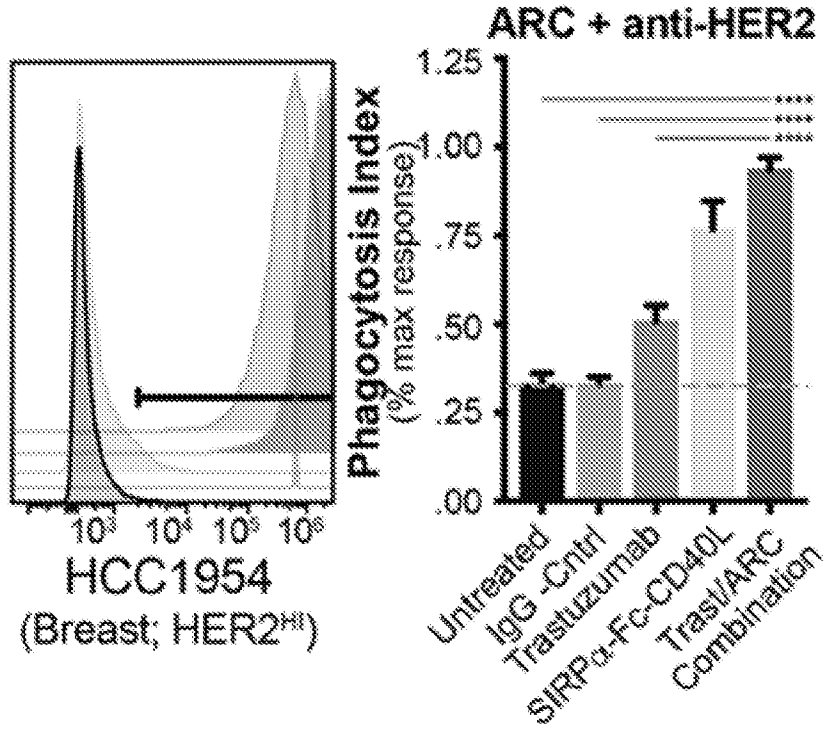


FIG. 14B

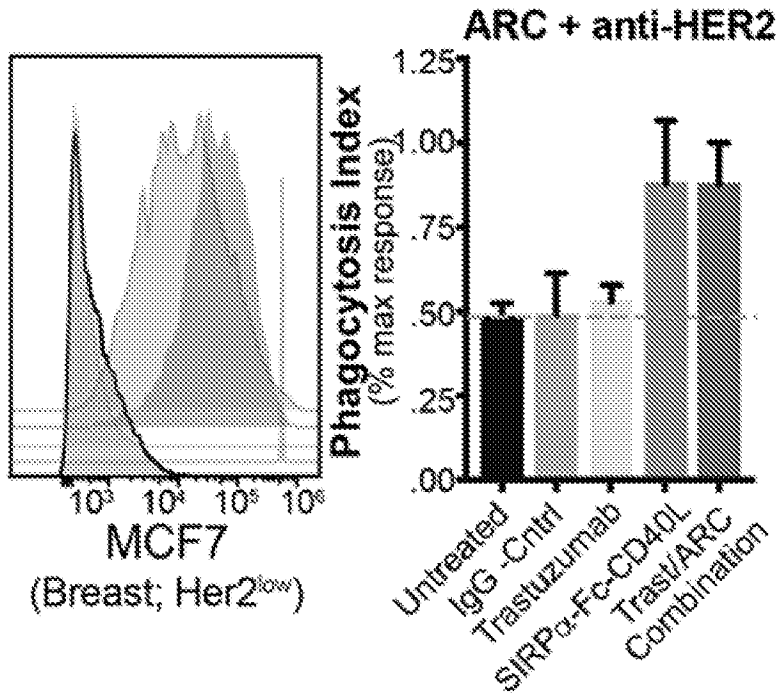


FIG. 15A

Reagent 1	Dose 1	Schedule 1		Reagent 2	Dose 2	Schedule 2	N=	# Rejection	% Survival @ Day 27
Vehicle	IP (PBS)	Days 8,11,13					8	**0**	**0**
Vehicle	IT (PBS)	Day 8					8	**0**	**0**
anti-CTLA4 (9D9)	100 µg	Days 8,11,13					8	**1**	**37.5**
DMXAA	100 µg	Day 8					8	**3**	**75**
				anti-PD1 (RMP1-14)	100µg	Days 11,13,15	8	**0**	**12.5**
				anti-OX40 (OX86)	100µg	Days 11,13,15	8	**1**	**37.5**
SIRPa-Fc-CD40L	300µg	Days 8,11,13					8	**0**	**50**
anti-CTLA4 (9D9)	100 µg	Days 8,11,13		anti-PD1	100µg	Days 13,15,17	8	**4**	**62.5**
anti-CTLA4 (9D9)	100 µg	Days 8,11,13		anti-OX40	100µg	Days 13,15,17	8	**1**	**62.5**
anti-CTLA4 (9D9)	100 µg	Days 8,11,13		SIRPa-Fc-CD40L	300µg	Days 13,15,17	8	**5**	**87.5**
SIRPa-Fc-CD40L	300µg	Days 8,11,13		anti-CTLA4 (9D9)	100 µg	Days 13,15,17	8	**1**	**62.5**
SIRPa-Fc-CD40L	300µg	Days 8,11,13		DMXAA	100µg	Day 13	8	**0**	**62.5**
DMXAA	100µg	Day 8		anti-PD1	100µg	Days 11,13,15	8	**4**	**75**
DMXAA	100µg	Day 8		anti-OX40	100µg	Days 11,13,15	8	**3**	**75**
DMXAA	100µg	Day 8		SIRPa-Fc-CD40L	300µg	Days 11,13,15	8	**3**	**75**

FIG. 15B

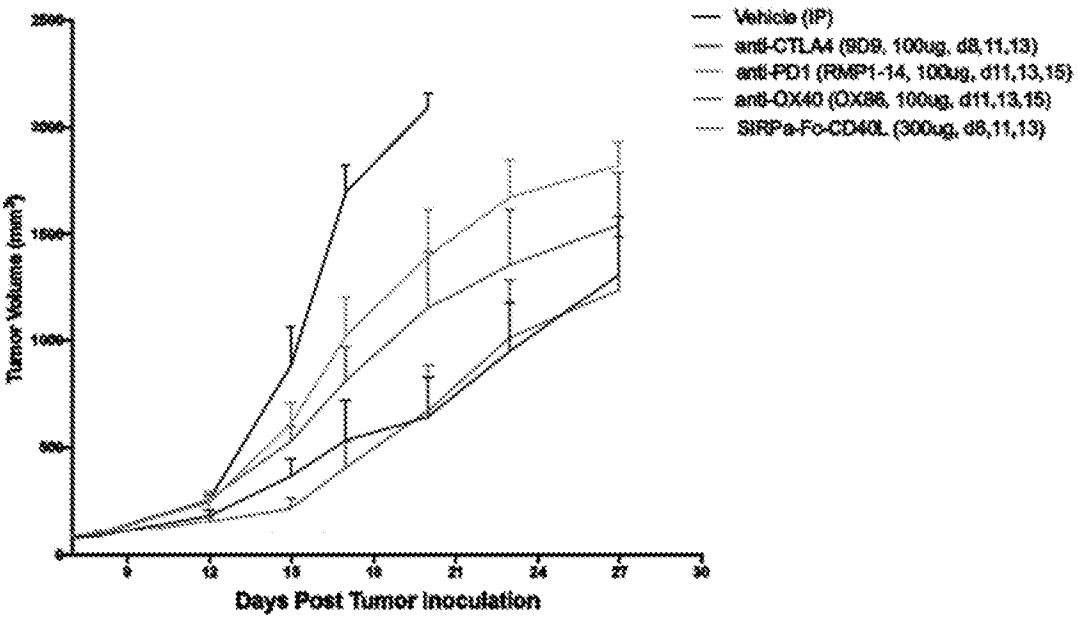


FIG. 15C

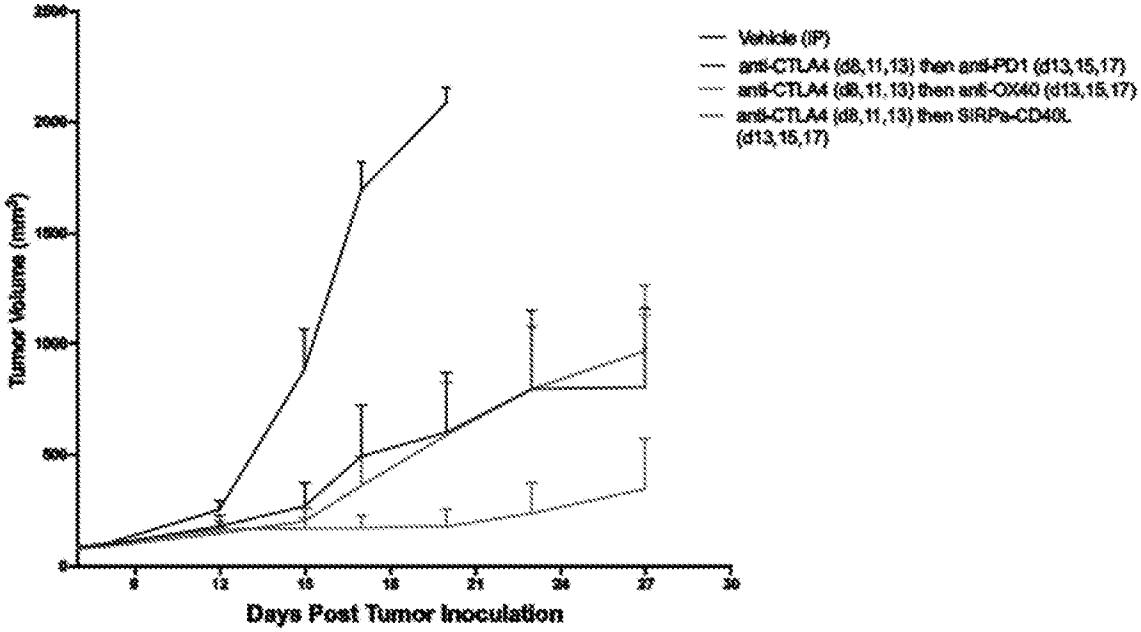


FIG. 15D

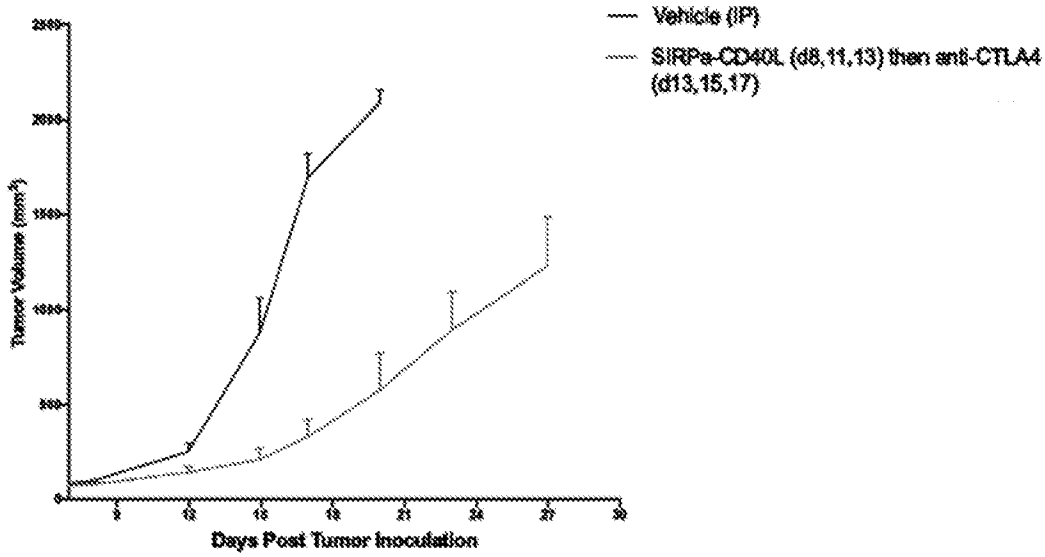

Vehicle (IP)
SIRPa-CD40L (d8,11,13) then anti-CTLA4 (d13,15,17)
Tumor Volume (mm³)
Days Post Tumor Inoculation

FIG. 16A

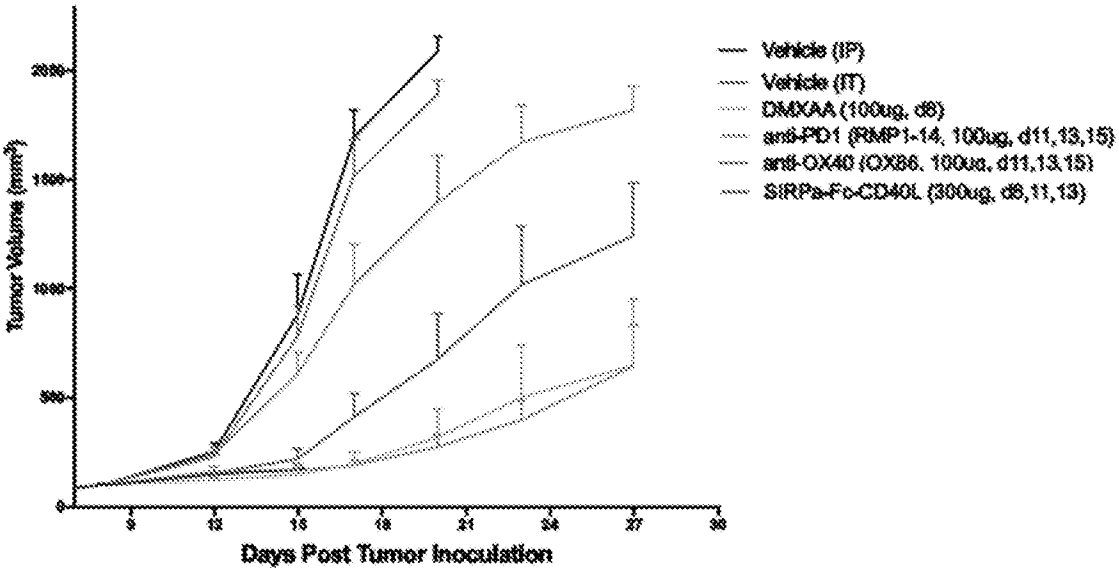


FIG. 16B

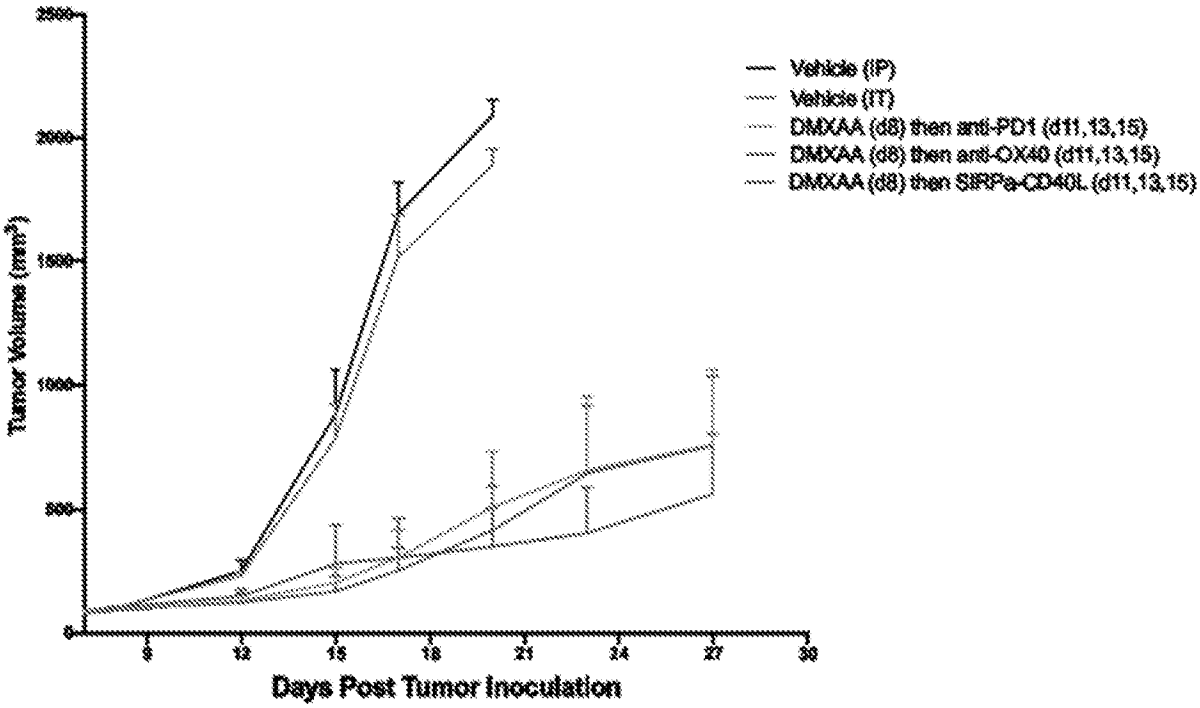


FIG. 17A

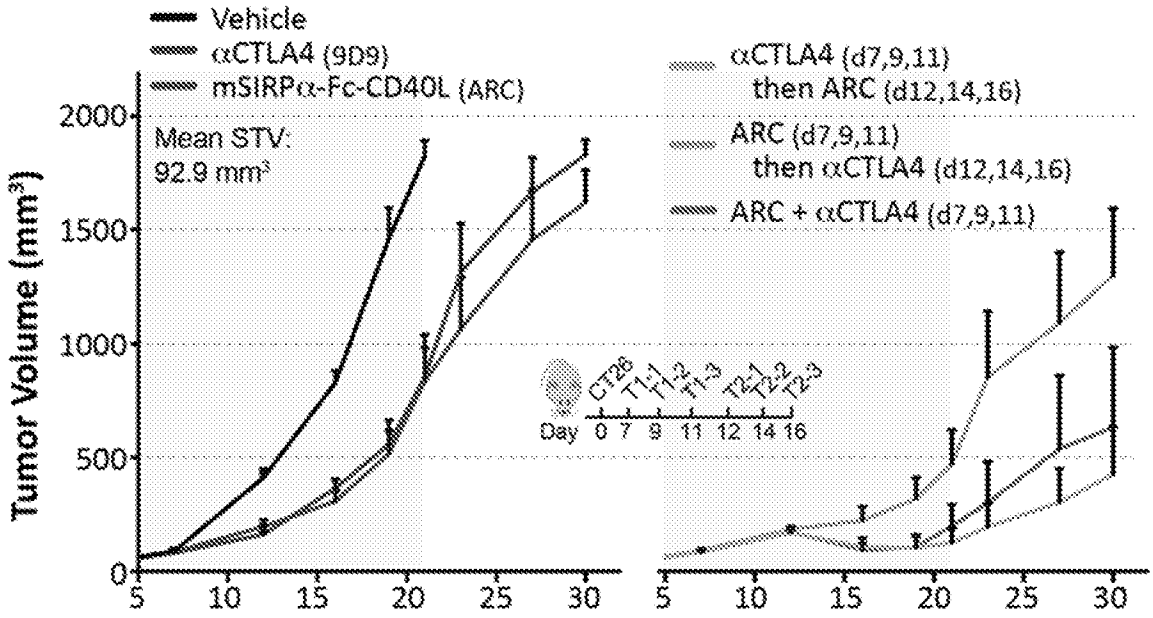


FIG. 17B

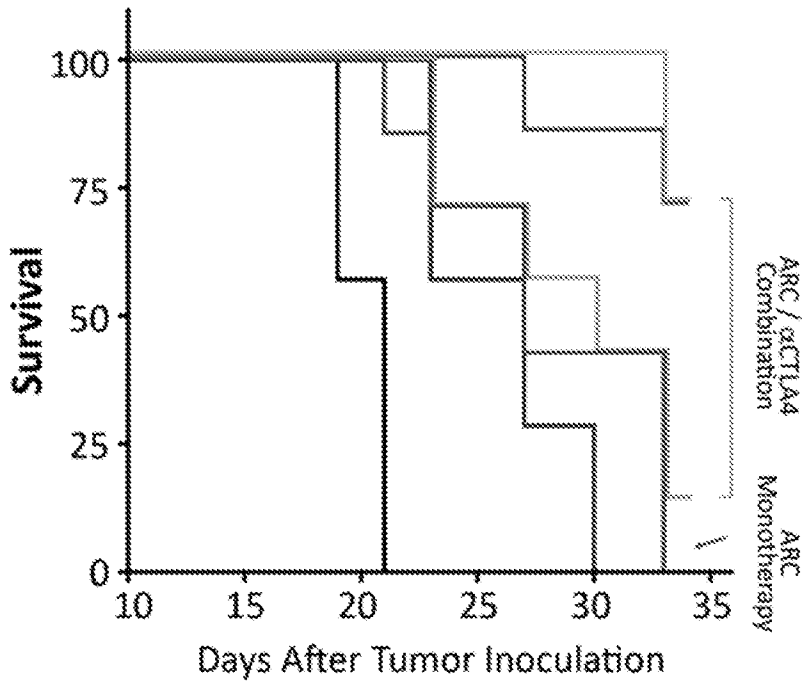


FIG. 17C

Monotherapy	primary	re-challenge	Tumor Rejection	Survival (day 35) Mantle Cox p-value vs. ARC Monotherapy
Vehicle	0/15	0/7		
αCTLA4 (9D9)	0/15	0/1		
mSIRPα-Fc-CD40L (ARC)	0/15	N/A		
Combinations				
αCTLA4 (d7,9,11) then ARC (d12,14,16)	9/15	7/9	→	.0027
ARC (d7,9,11) then αCTLA4 (d12,14,16)	3/15	2/3	→	.6228
ARC + αCTLA4 (d7,9,11)	4/7	4/4	→	.0072

FIG. 18A

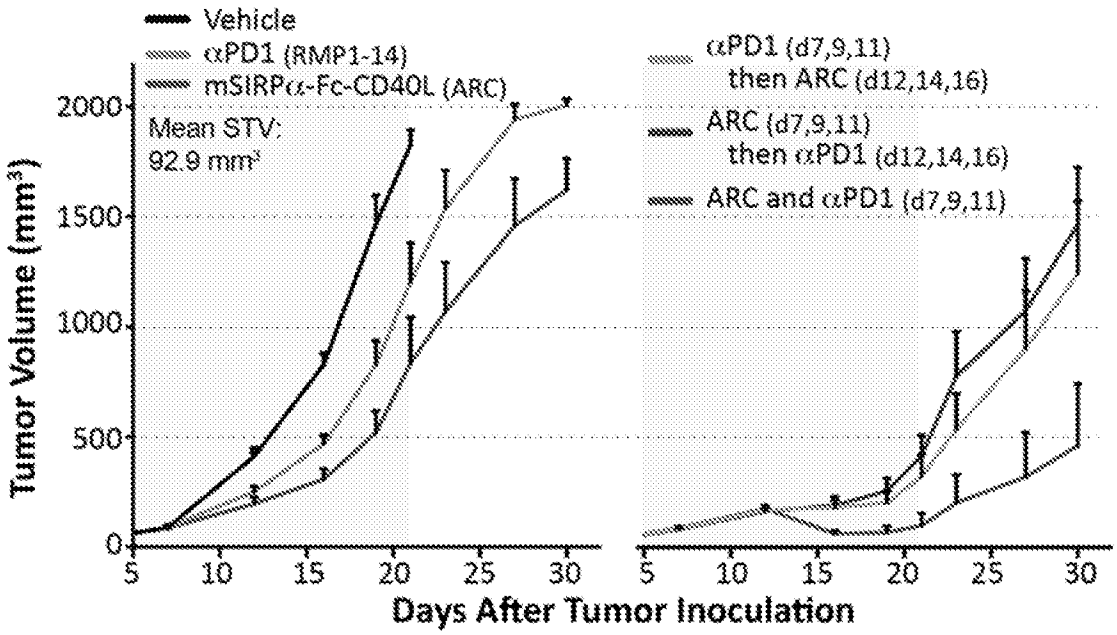


FIG. 18B

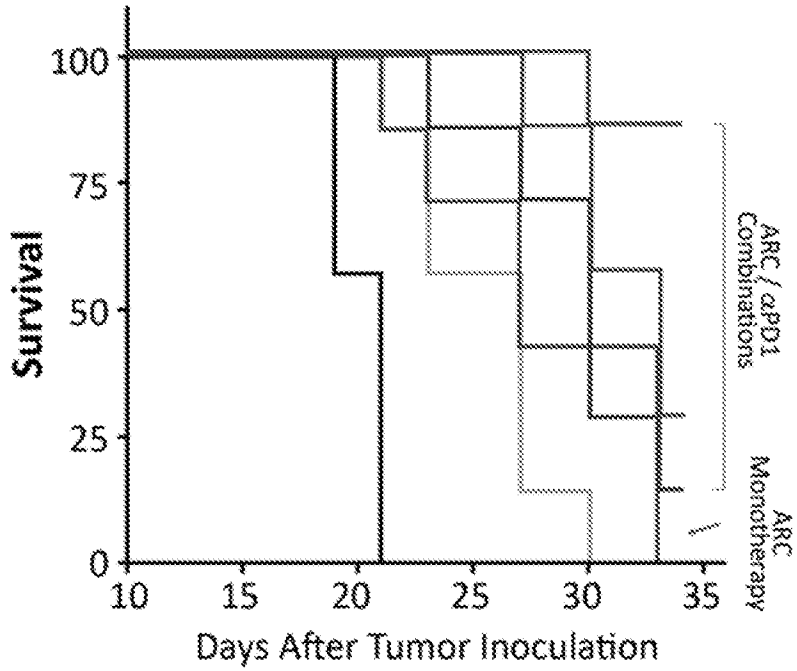


FIG. 18C

		primary	re-challenge	Tumor Rejection	Survival (day 35) Mantle Cox p-value vs. ARC Monotherapy
Monotherapy					
▬	Vehicle	0/15	0/7		
▬	αPD1 (RMP1-14)	0/7	N/A		
▬	mSIRPα-Fc-CD40L (ARC)	0/15	N/A		
Combinations					
▬	αPD1 (d7,9,11) then ARC (d12,14,16)	1/7	1/1		→ .1503
▬	ARC (d7,9,11) then αPD1 (d12,14,16)	1/7	1/1		→ .6301
▬	ARC and αPD1 (d7,9,11)	3/7	3/3		→ .0024

FIG. 19

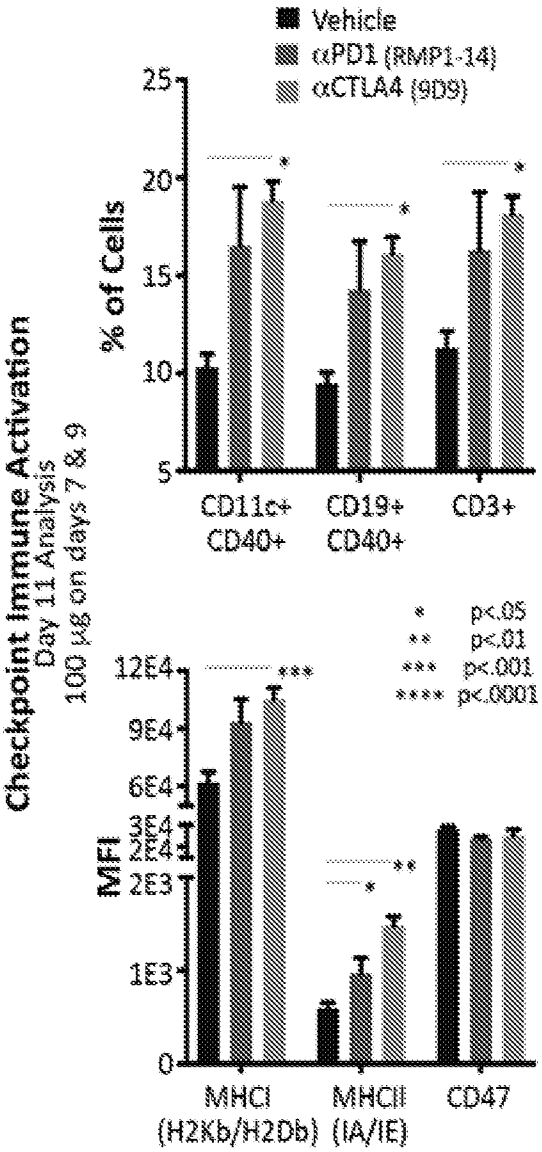

Checkpoint Immune Activation
Day 11 Analysis
100 µg on days 7 & 9
Vehicle
αPD1 (RMP1-14)
αCTLA4 (9D9)
% of Cells
25
20
15
10
5
CD11c+ CD40+
CD19+ CD40+
CD3+
* p<.05
** p<.01
*** p<.001
**** p<.0001
MFI
12E4
9E4
6E4
2E3
1E3
0
MHCI (H2Kb/H2Db)
MHCII (IA/IE)
CD47

FIG. 20A

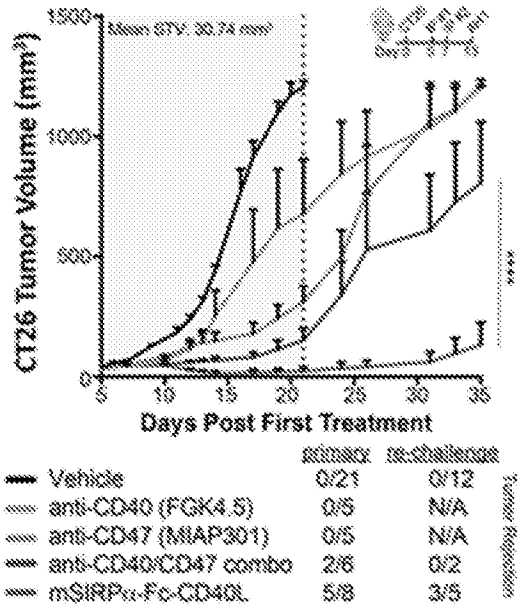


FIG. 20B

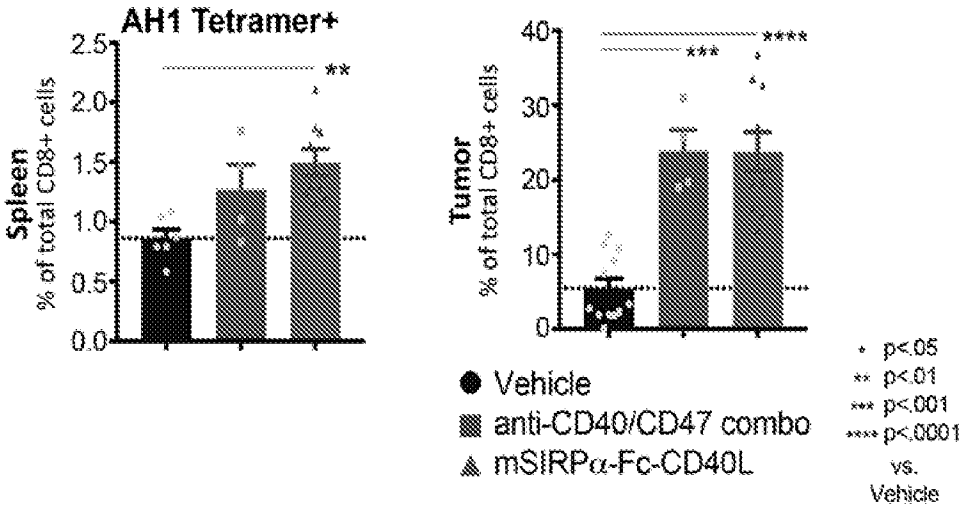


FIG. 20C

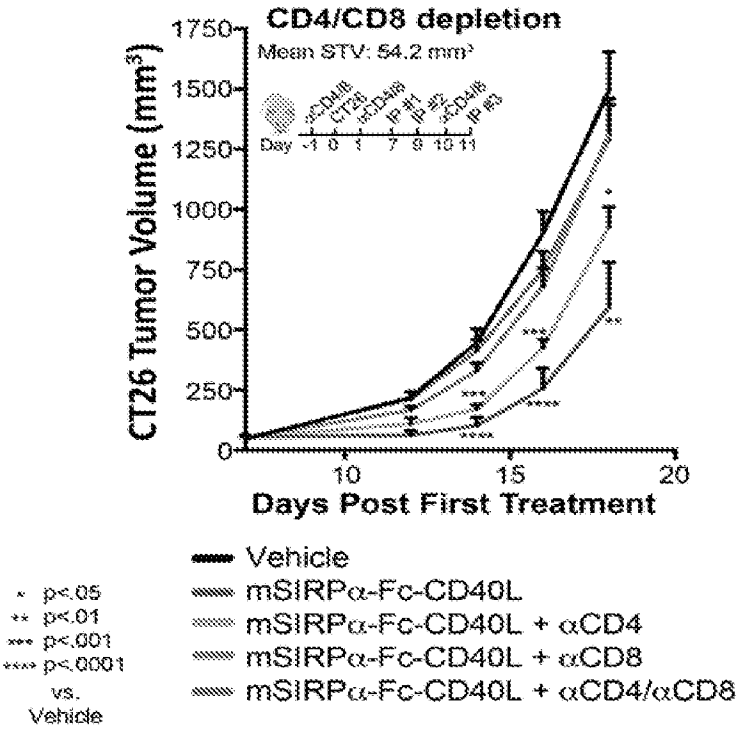


FIG. 20D

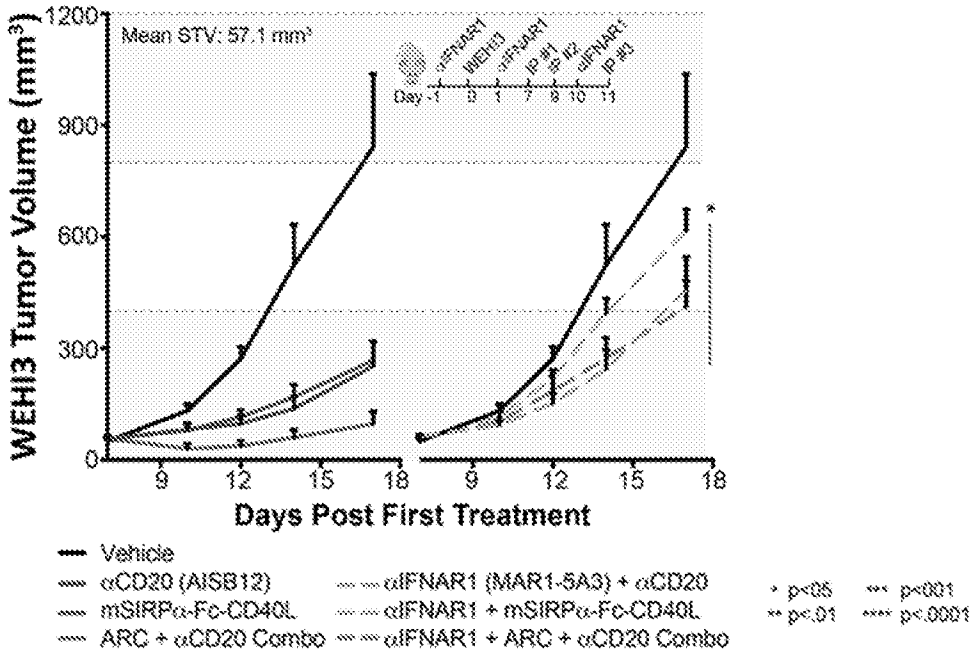


FIG. 20E

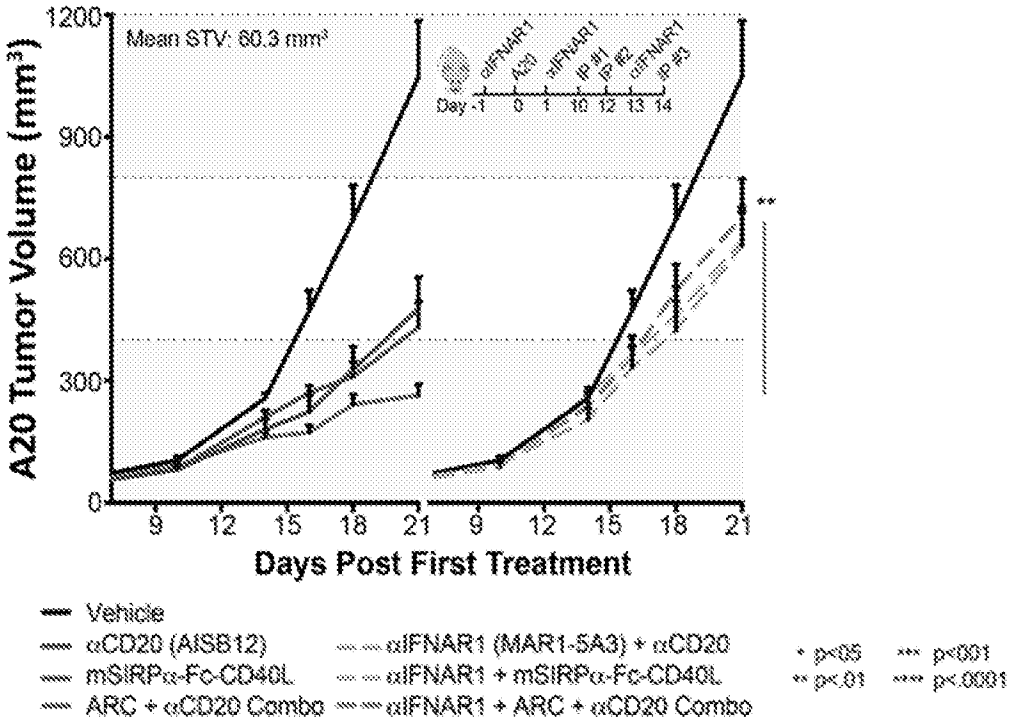


FIG. 20F

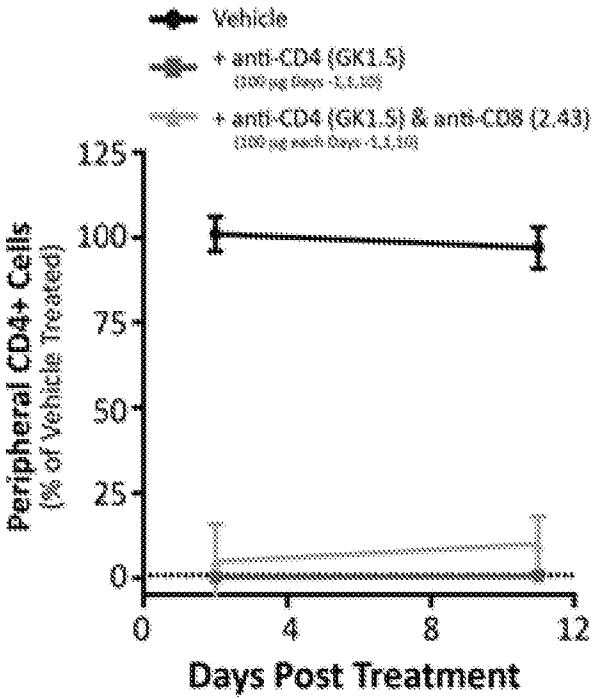


FIG. 20G

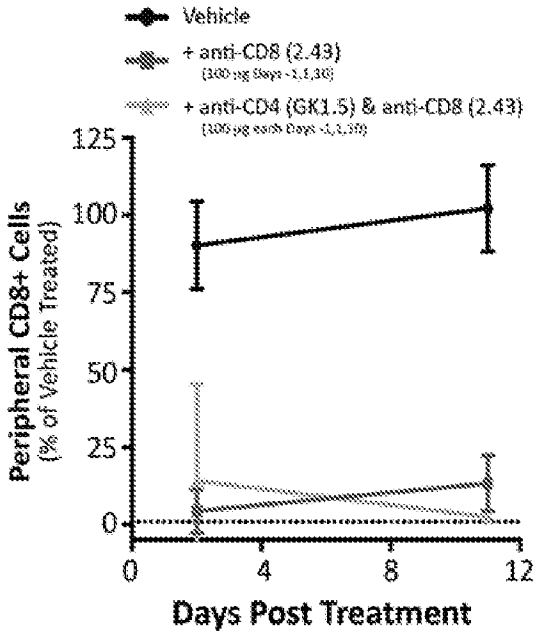


FIG. 20H

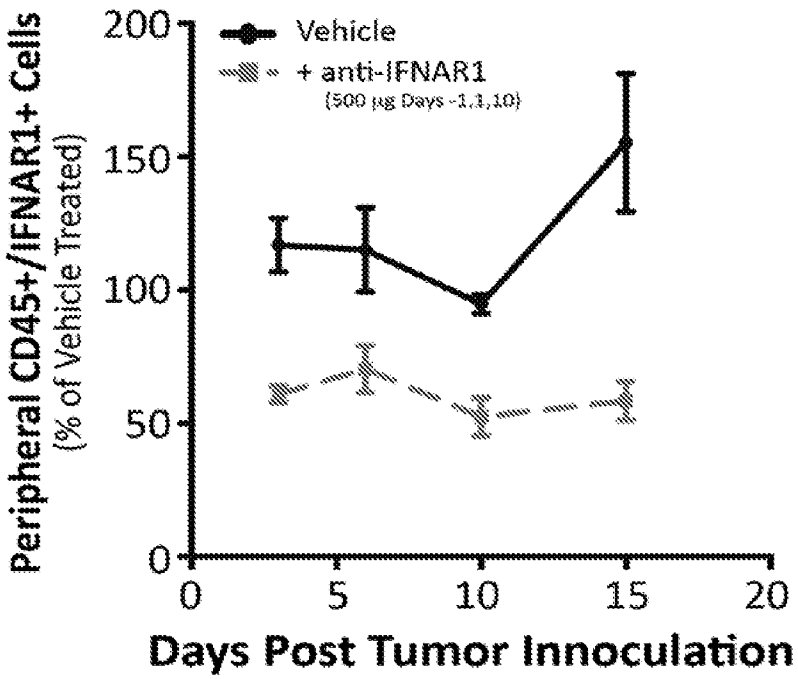


FIG. 21

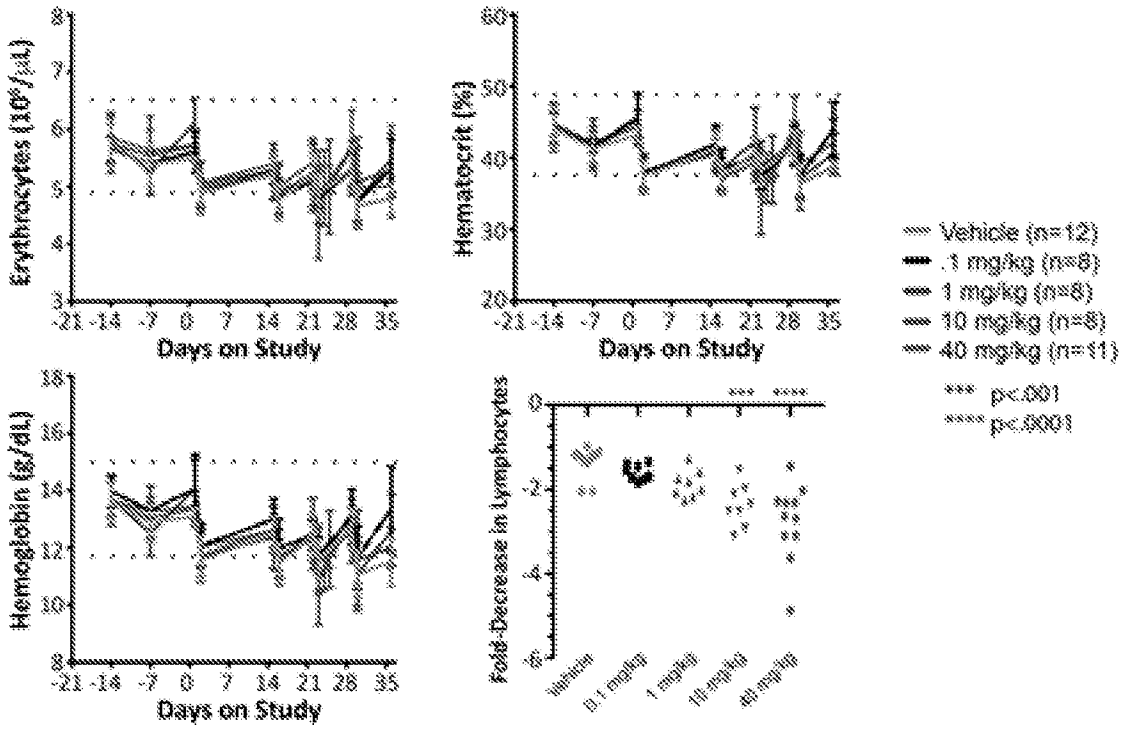


FIG. 22A

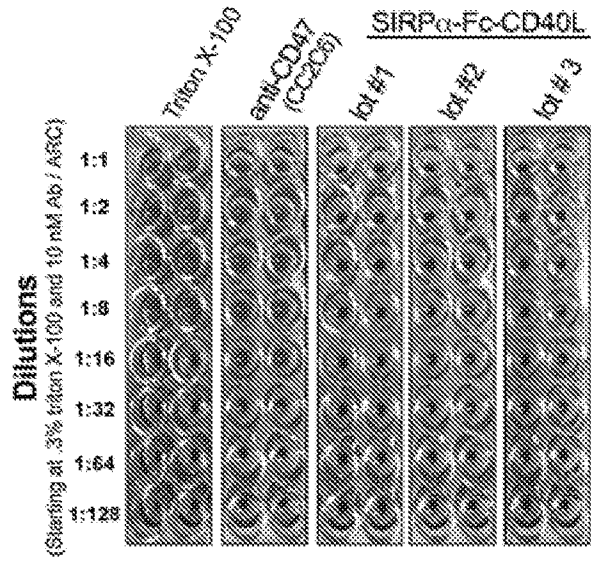


FIG. 22B

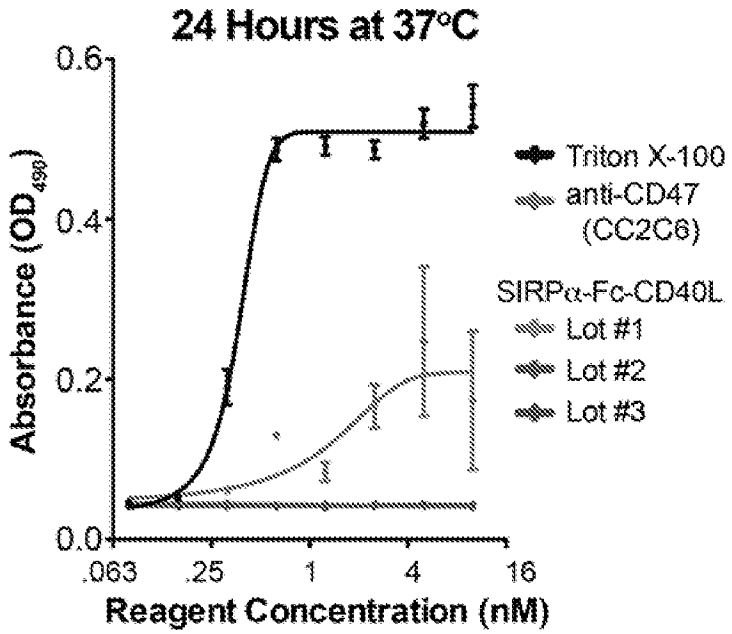


FIG. 22C

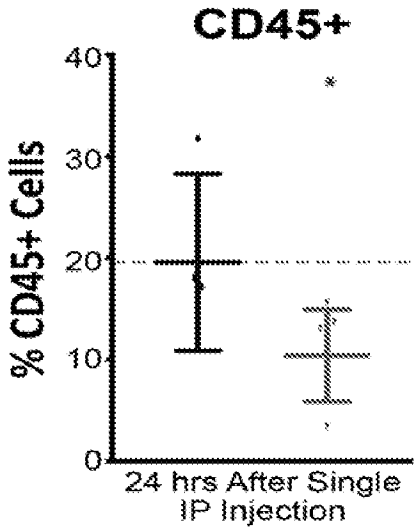


FIG. 22D

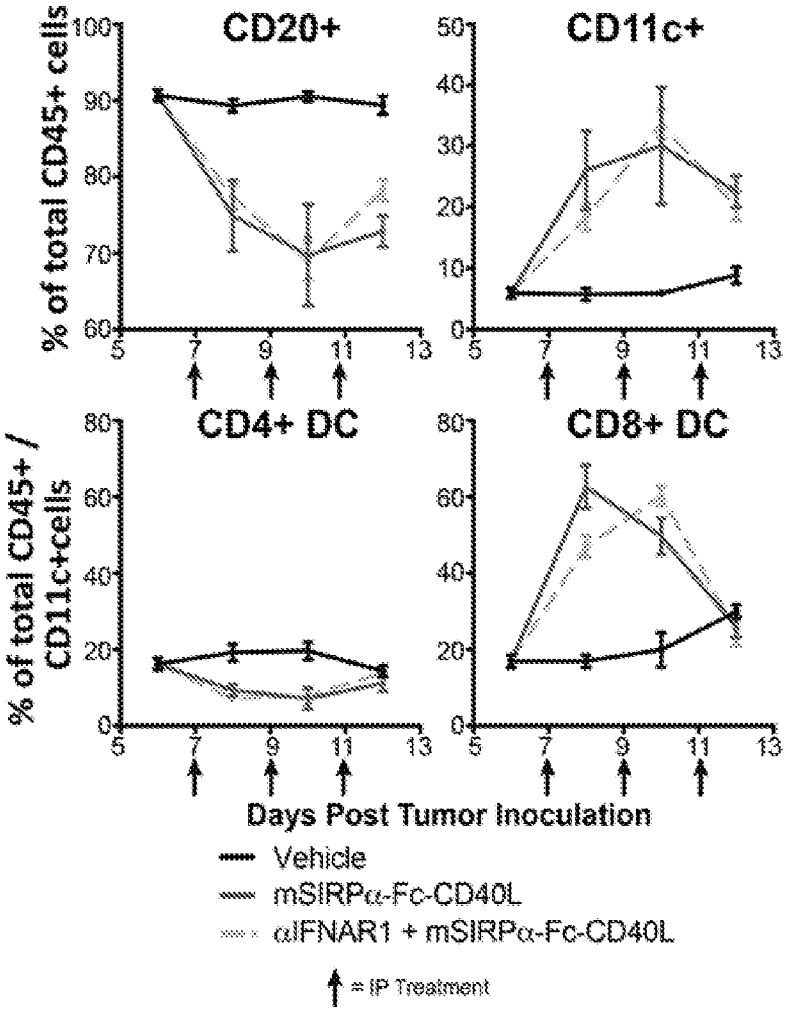

CD20+
CD11c+
CD4+ DC
CD8+ DC
% of total CD45+ cells
% of total CD45+ / CD11c+cells
Days Post Tumor Inoculation
Vehicle
mSIRPα-Fc-CD40L
αIFNAR1 + mSIRPα-Fc-CD40L
= IP Treatment

FIG. 23A

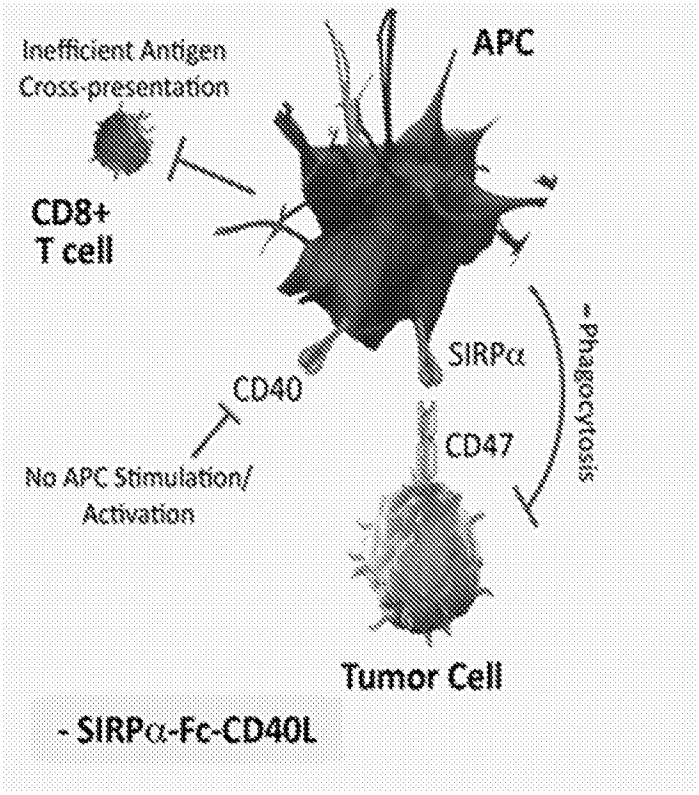


FIG. 23B

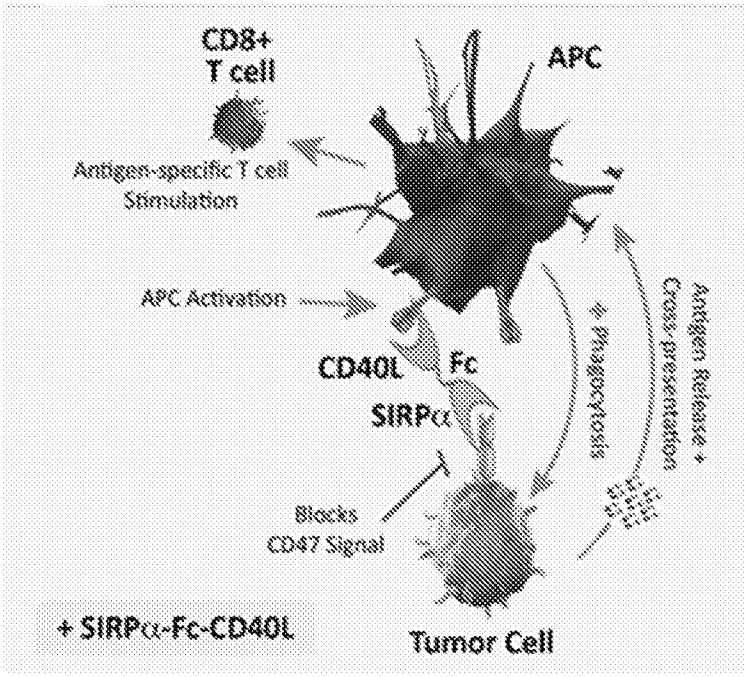


FIG. 23C

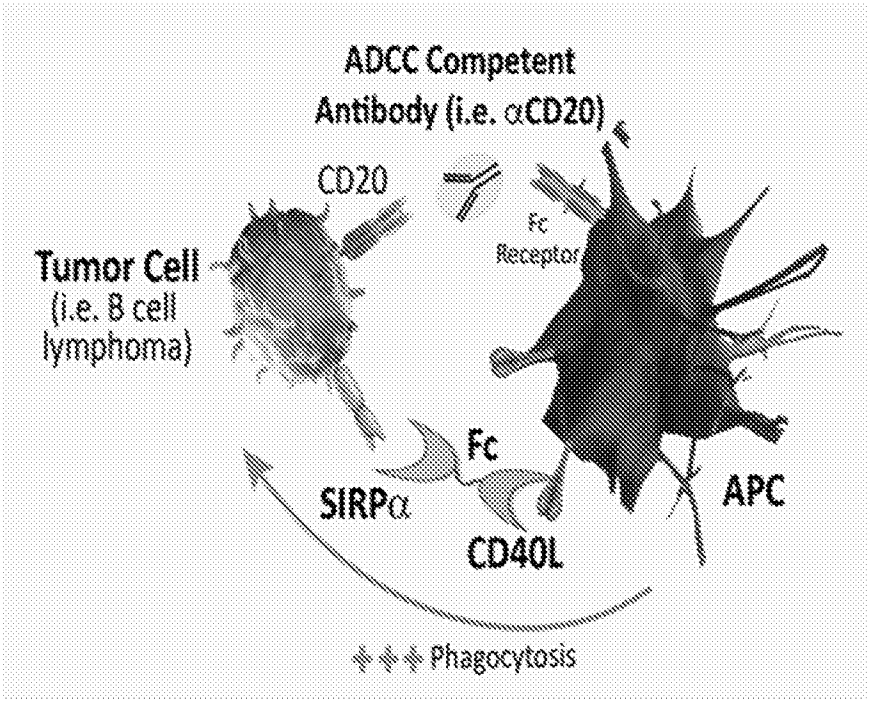


FIG. 24

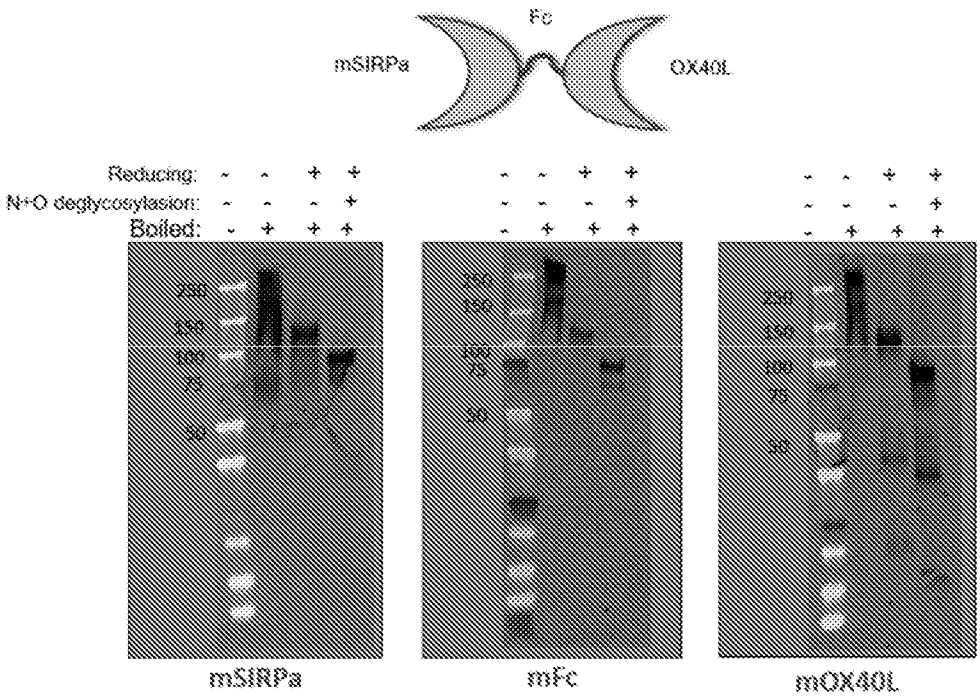
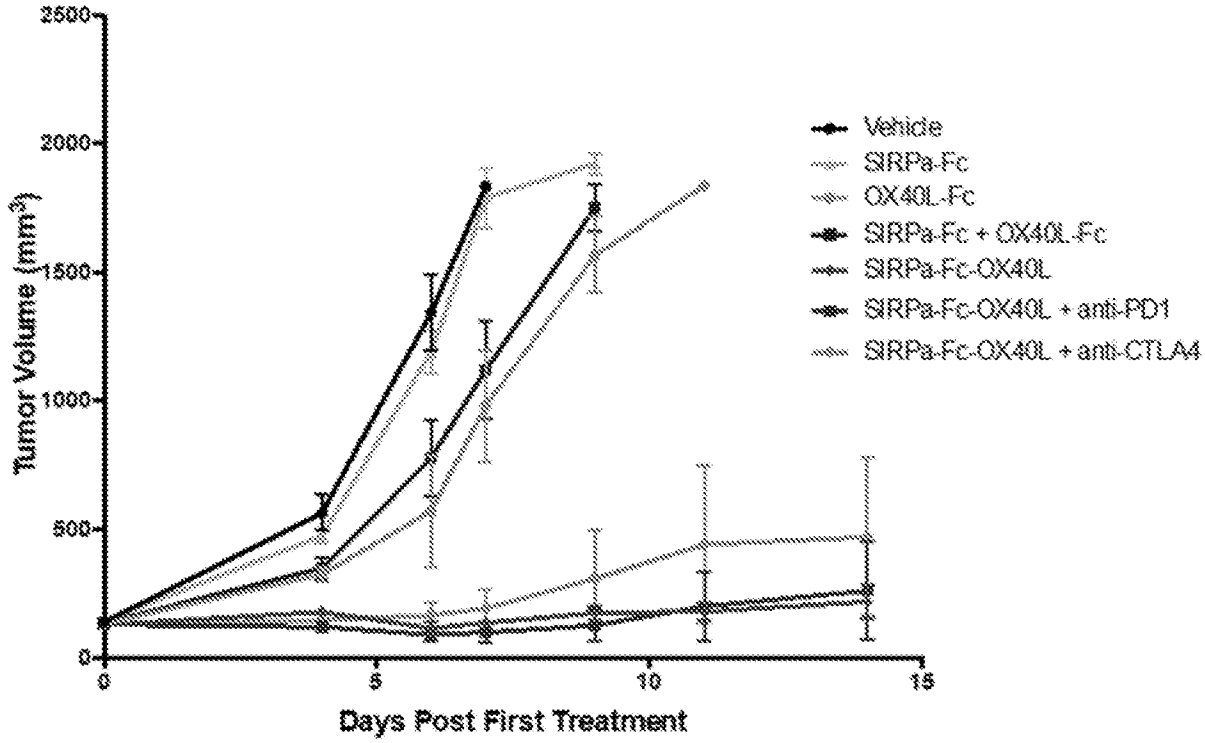


FIG. 25A


Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
5
10
15
Days Post First Treatment
Vehicle
SIRPa-Fc
OX40L-Fc
SIRPa-Fc + OX40L-Fc
SIRPa-Fc-OX40L
SIRPa-Fc-OX40L + anti-PD1
SIRPa-Fc-OX40L + anti-CTLA4

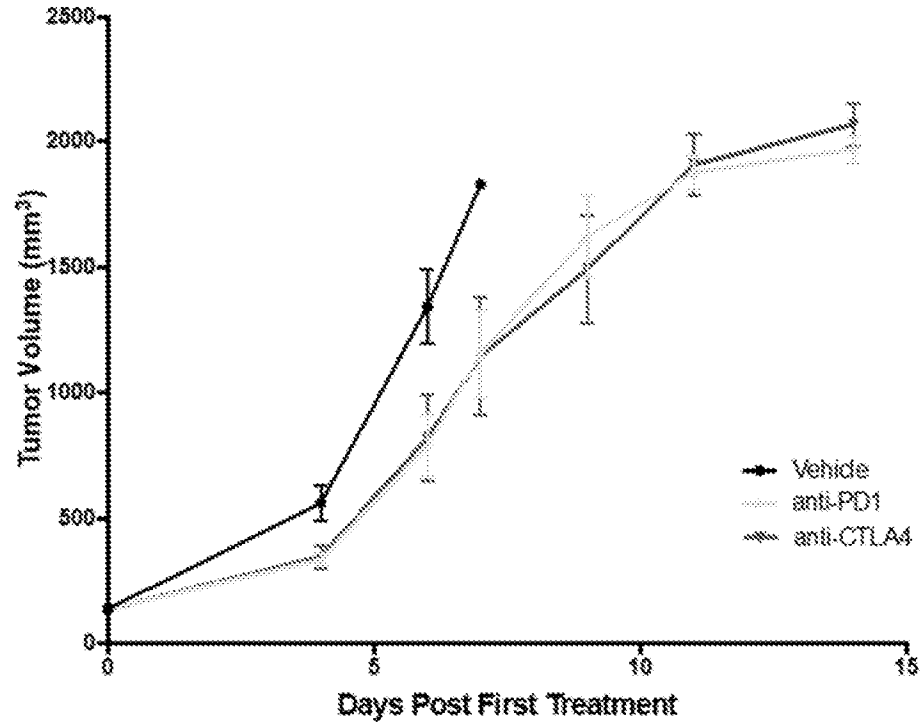

Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
5
10
15
Days Post First Treatment
Vehicle
anti-PD1
anti-CTLA4

FIG. 25B

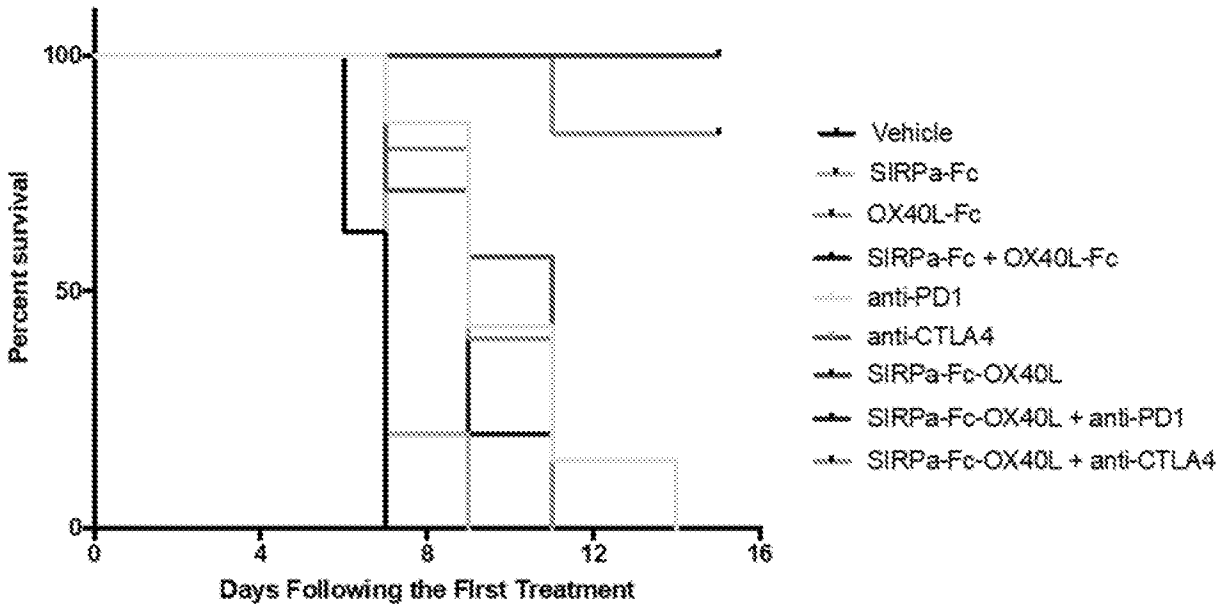


FIG. 25C

			Group Size	Primary Tumor Rejection		Survival %
Group	**Dose (μg)**	**Schedule (days)**	**(n=)**	**#**	**%**	**at day 14**
Vehicle			8	0/8	0	0
SIRPa-Fc	150	0,2,4	5	0/5	0	0
Fc-OX40L	150	0,2,4	5	0/5	0	0
SIRPa-Fc + Fc-OX40L	150/150	0,2,4	5	0/5	0	0
anti-PD1 (RMP1-14)	100	0,2,4	7	0/7	0	0
anti-CTLA4 (9D9)	100	0,2,4	7	0/7	0	0
SIRPa-Fc-OX40L	300	0,2,4	6	0/6	0	100
SIRPa-Fc-OX40L + anti-PD1	300/100	0,2,4	6	3/6	50	100
SIRPa-Fc-OX40L + anti-CTLA4	300/100	0,2,4	6	2/6	33	83

FIG. 26A

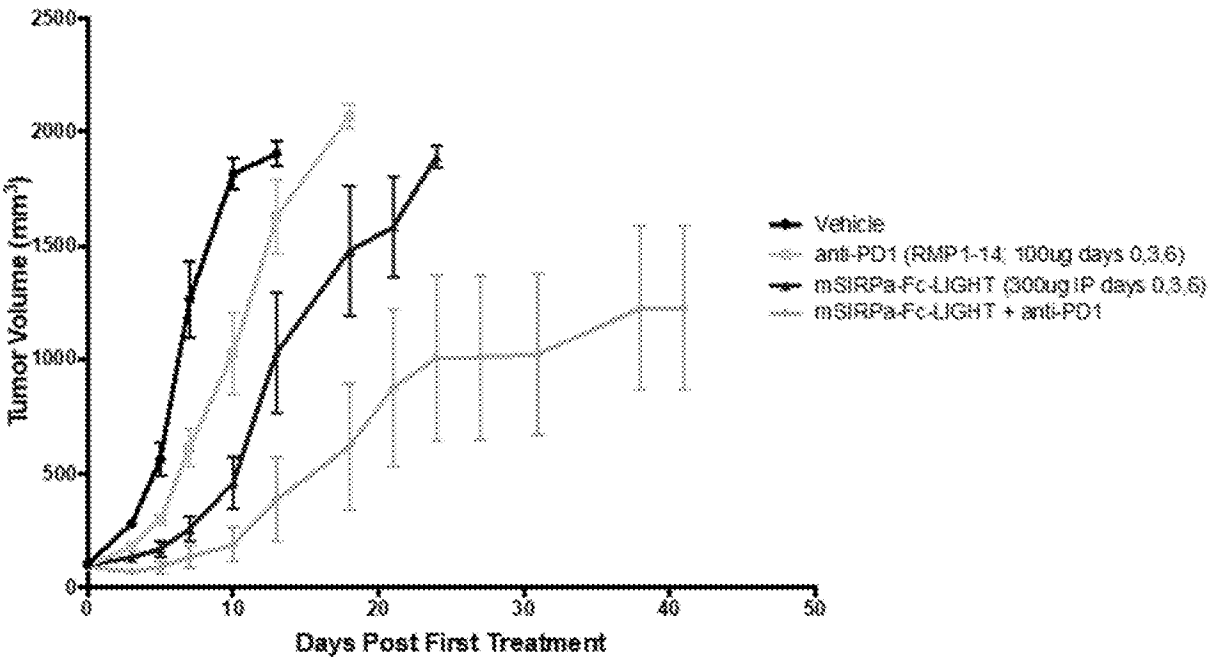


FIG. 26B

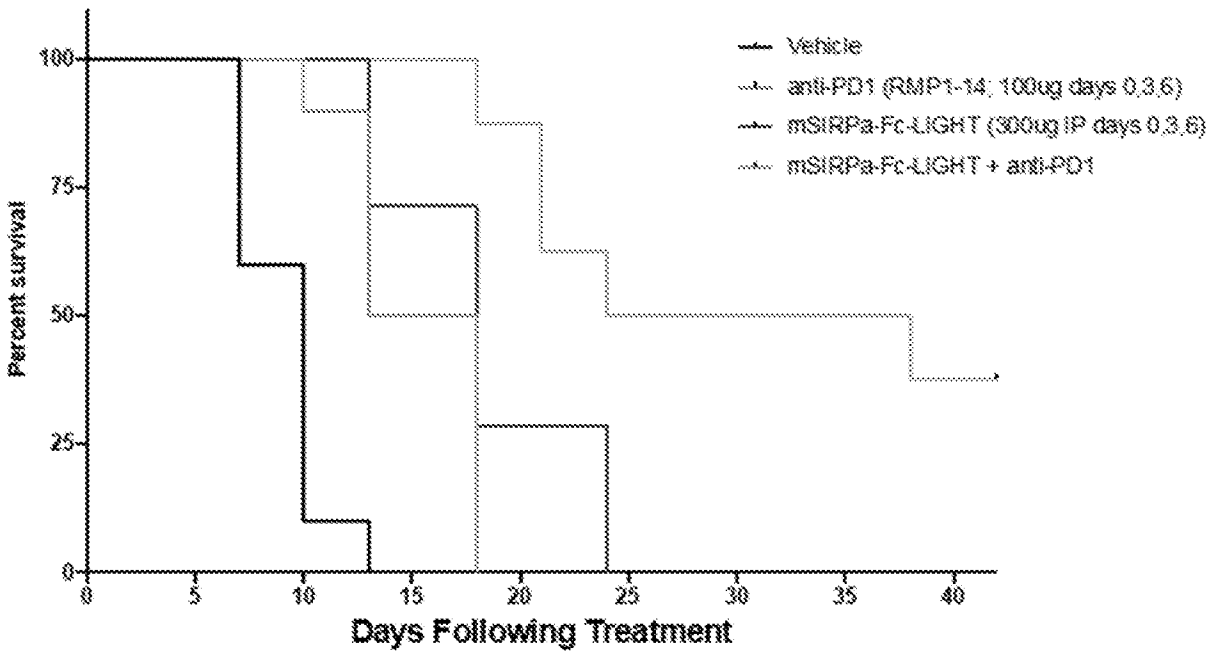


FIG. 26C

Group	Dose (µg)	Schedule (days)	Monotherapy (n=)	Combination (n=)
Vehicle	PBS	0, 3, 6	10	N/A
anti-PD1 (clone RMP1-14)	100	0, 3, 6	10	N/A
SIRPa-Fc-LIGHT	300	0, 3, 6	7	8

FIG. 26D

ARC	Tumor Volume Day 0 to Day 10 ARC vs ARC/aPD1 Combo		Tumor Volume Day 0 to Day 10 ARC/aPD1 vs aPD1		Tumor Rejection Primary		Tumor Rejection Secondary		Survival Alive at day 24	Survival ARC vs ARC/aPD1
	Fold Change	p-value	Fold Change	p-value	#	%	#	%	%	p-value
SIRPa-Fc-LIGHT	3.96	0.0251	8.58	<.0001	0/7	0	0/0	0	28.6	0.0187
SIRPa-Fc-LIGHT + aPD1					3/8	37.5	2/3	66.6	62.5	

SEQUENCE LISTING

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<120> COMBINATION THERAPIES COMPRISING SIRP ALPHA-BASED CHIMERIC PROTEINS

<130> SHK-013PC

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            20                  25                  30


Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
        35                  40                  45


Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    50                  55                  60


Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65                  70                  75                  80


Gln Asp Trp Leu Ser Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys
                85                  90                  95


Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln
            100                 105                 110


Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met
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```
        115                 120                 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    130                 135                 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145                 150                 155                 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                165                 170                 175

Tyr Ser Arg Leu Thr Val Asp Lys Ser Ser Trp Gln Glu Gly Asn Val
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Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
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Pro Lys Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            20                  25                  30

Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
        35                  40                  45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    50                  55                  60
```

```
Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Thr Pro His
65                  70                  75                  80

Ser Asp Trp Leu Ser Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys
                85                  90                  95

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln
            100                 105                 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met
        115                 120                 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    130                 135                 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145                 150                 155                 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                165                 170                 175

Tyr Ser Arg Leu Thr Val Asp Lys Ser Ser Trp Gln Glu Gly Asn Val
            180                 185                 190

Phe Ser Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln
        195                 200                 205

Lys Ser Leu Ser Leu Ser Leu Gly Lys
    210                 215


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```

```
1               5                   10                  15

Pro Lys Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            20                  25                  30

Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
        35                  40                  45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    50                  55                  60

Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65                  70                  75                  80

Gln Asp Trp Leu Ser Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys
                85                  90                  95

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln
            100                 105                 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met
        115                 120                 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    130                 135                 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145                 150                 155                 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                165                 170                 175

Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val
            180                 185                 190

Phe Ser Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln
        195                 200                 205

Lys Ser Leu Ser Leu Ser Leu Gly Lys
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1 5 10 15

Gly Gly Gly Ser
20

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1   5   10   15

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            20   25

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<223> Synthetic Polypeptide

<400> 30

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1   5   10   15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
            20   25   30

<210> 31
<211> 35
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 31

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1   5   10   15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
            20   25   30

Gly Gly Ser
        35

<210> 32
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 32

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser
35 40

<210> 33
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 33

Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 34
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 34

Gly Gly Gly Gly Gly Gly Gly Gly
1 5

<210> 35
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 35

Gly Gly Gly Gly Gly Gly
1 5

<210> 36
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 36

Glu Ala Ala Ala Lys
1 5

<210> 37
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 37

Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10

<210> 38
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 38

Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10 15

<210> 39
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 39

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala
1 5 10

<210> 40
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 40

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10 15

Ala

<210> 41
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 41

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10 15

Glu Ala Ala Ala Lys Ala
20

<210> 42
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 42

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10 15

Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala
20 25

<210> 43
<211> 46
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 43

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10 15

Glu Ala Ala Ala Lys Ala Leu Glu Ala Glu Ala Ala Ala Lys Glu Ala
20 25 30

Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala
35 40 45

<210> 44
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 44

Pro Ala Pro Ala Pro
1 5

<210> 45
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 45

Lys Glu Ser Gly Ser Val Ser Ser Glu Gln Leu Ala Gln Phe Arg Ser
1 5 10 15

Leu Asp

<210> 46
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 46

Gly Ser Ala Gly Ser Ala Ala Gly Ser Gly Glu Phe
1 5 10

<210> 47
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 47

Gly Gly Gly Ser Glu
1 5

<210> 48
<211> 5

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 48

Gly Ser Glu Ser Gly
1 5

<210> 49
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 49

Gly Ser Glu Gly Ser
1 5

<210> 50
<211> 35
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 50

Gly Glu Gly Gly Ser Gly Glu Gly Ser Ser Gly Glu Gly Ser Ser Ser
1 5 10 15

Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu
20 25 30

Gly Gly Ser
35

<210> 51
<211> 234
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 51

```
Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu
1               5                  10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30


Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45


Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60


Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80


Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Ser
                85                  90                  95


Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys Gly Leu Pro Ser Ser
            100                 105                 110


Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125


Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140


Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160


Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175


Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
            180                 185                 190
```

```
Val Asp Lys Ser Ser Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205


Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220


Ser Leu Gly Lys Ile Glu Gly Arg Met Asp
225                 230


<210>  52
<211>  234
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Polypeptide

<400>  52

Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu
1               5                   10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu
            20                  25                  30


Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45


Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60


Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80


Tyr Arg Val Val Ser Val Leu Thr Thr Pro His Ser Asp Trp Leu Ser
                85                  90                  95


Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys Gly Leu Pro Ser Ser
            100                 105                 110
```

```
Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125


Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140


Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160


Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175


Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
            180                 185                 190


Val Asp Lys Ser Ser Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205


Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220


Ser Leu Gly Lys Ile Glu Gly Arg Met Asp
225                 230


<210>  53
<211>  234
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Polypeptide

<400>  53

Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu
1               5                   10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu
            20                  25                  30


Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45
```

```
Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Ser
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys Gly Leu Pro Ser Ser
            100                 105                 110

Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Leu Gly Lys Ile Glu Gly Arg Met Asp
225                 230
```

<210> 54
<211> 234

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 54

```
Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu
1               5                  10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30


Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45


Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60


Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80


Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Ser
                85                  90                  95


Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys Gly Leu Pro Ser Ser
            100                 105                 110


Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125


Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140


Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160


Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175
```

```
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
            180                 185                 190


Val Asp Lys Ser Ser Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205


Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220


Ser Leu Gly Lys Ile Glu Gly Arg Met Asp
225                 230


<210>  55
<211>  234
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Polypeptide

<400>  55

Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu
1               5                   10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu
            20                  25                  30


Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45


Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60


Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80


Tyr Arg Val Val Ser Val Leu Thr Thr Pro His Ser Asp Trp Leu Ser
                85                  90                  95


Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys Gly Leu Pro Ser Ser
            100                 105                 110
```

```
Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125


Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140


Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160


Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175


Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
            180                 185                 190


Val Asp Lys Ser Ser Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205


Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220


Ser Leu Gly Lys Ile Glu Gly Arg Met Asp
225                 230


<210>  56
<211>  234
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Polypeptide

<400>  56

Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu
1               5                   10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu
            20                  25                  30
```

```
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser
        35                  40                  45


Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60


Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80


Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Ser
                85                  90                  95


Gly Lys Glu Tyr Lys Cys Lys Val Ser Ser Lys Gly Leu Pro Ser Ser
            100                 105                 110


Ile Glu Lys Thr Ile Ser Asn Ala Thr Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125


Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140


Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160


Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175


Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
            180                 185                 190


Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205


Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220


Ser Leu Gly Lys Ile Glu Gly Arg Met Asp
225                 230
```

<210> 57
<211> 343
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 57

```
Glu Glu Glu Leu Gln Val Ile Gln Pro Asp Lys Ser Val Leu Val Ala
1               5                  10                  15


Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ala Thr Ser Leu Ile Pro
            20                  25                  30


Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro Gly Arg Glu Leu
        35                  40                  45


Ile Tyr Asn Gln Lys Glu Gly His Phe Pro Arg Val Thr Thr Val Ser
    50                  55                  60


Asp Leu Thr Lys Arg Asn Asn Met Asp Phe Ser Ile Arg Ile Gly Asn
65                  70                  75                  80


Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Val Lys Phe Arg Lys
                85                  90                  95


Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala Gly Thr Glu Leu
            100                 105                 110


Ser Val Arg Ala Lys Pro Ser Ala Pro Val Val Ser Gly Pro Ala Ala
        115                 120                 125


Arg Ala Thr Pro Gln His Thr Val Ser Phe Thr Cys Glu Ser His Gly
    130                 135                 140


Phe Ser Pro Arg Asp Ile Thr Leu Lys Trp Phe Lys Asn Gly Asn Glu
145                 150                 155                 160


Leu Ser Asp Phe Gln Thr Asn Val Asp Pro Val Gly Glu Ser Val Ser
                165                 170                 175
```

```
Tyr Ser Ile His Ser Thr Ala Lys Val Val Leu Thr Arg Glu Asp Val
            180                 185                 190

His Ser Gln Val Ile Cys Glu Val Ala His Val Thr Leu Gln Gly Asp
        195                 200                 205

Pro Leu Arg Gly Thr Ala Asn Leu Ser Glu Thr Ile Arg Val Pro Pro
    210                 215                 220

Thr Leu Glu Val Thr Gln Gln Pro Val Arg Ala Glu Asn Gln Val Asn
225                 230                 235                 240

Val Thr Cys Gln Val Arg Lys Phe Tyr Pro Gln Arg Leu Gln Leu Thr
                245                 250                 255

Trp Leu Glu Asn Gly Asn Val Ser Arg Thr Glu Thr Ala Ser Thr Val
            260                 265                 270

Thr Glu Asn Lys Asp Gly Thr Tyr Asn Trp Met Ser Trp Leu Leu Val
        275                 280                 285

Asn Val Ser Ala His Arg Asp Asp Val Lys Leu Thr Cys Gln Val Glu
    290                 295                 300

His Asp Gly Gln Pro Ala Val Ser Lys Ser His Asp Leu Lys Val Ser
305                 310                 315                 320

Ala His Pro Lys Glu Gln Gly Ser Asn Thr Ala Ala Glu Asn Thr Gly
                325                 330                 335

Ser Asn Glu Arg Asn Ile Tyr
            340
```

<210> 58
<211> 215
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 58

```
His Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp
1               5                  10                  15


Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser
            20                  25                  30


Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe
        35                  40                  45


Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser
    50                  55                  60


Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val
65                  70                  75                  80


Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu
                85                  90                  95


Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly
            100                 105                 110


Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
        115                 120                 125


Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
    130                 135                 140


Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu
145                 150                 155                 160


Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser
                165                 170                 175


Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe
            180                 185                 190
```

```
Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr
        195                 200                 205


Ser Phe Gly Leu Leu Lys Leu
    210                 215


<210>  59
<211>  133
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Sequence

<400>  59

Gln Val Ser His Arg Tyr Pro Arg Ile Gln Ser Ile Lys Val Gln Phe
1               5                   10                  15


Thr Glu Tyr Lys Lys Glu Lys Gly Phe Ile Leu Thr Ser Gln Lys Glu
            20                  25                  30


Asp Glu Ile Met Lys Val Gln Asn Asn Ser Val Ile Ile Asn Cys Asp
        35                  40                  45


Gly Phe Tyr Leu Ile Ser Leu Lys Gly Tyr Phe Ser Gln Glu Val Asn
    50                  55                  60


Ile Ser Leu His Tyr Gln Lys Asp Glu Glu Pro Leu Phe Gln Leu Lys
65                  70                  75                  80


Lys Val Arg Ser Val Asn Ser Leu Met Val Ala Ser Leu Thr Tyr Lys
                85                  90                  95


Asp Lys Val Tyr Leu Asn Val Thr Thr Asp Asn Thr Ser Leu Asp Asp
            100                 105                 110


Phe His Val Asn Gly Gly Glu Leu Ile Leu Ile His Gln Asn Pro Gly
        115                 120                 125


Glu Phe Cys Val Leu
    130
```

<210> 60
<211> 792
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 60

```
Glu Glu Glu Leu Gln Val Ile Gln Pro Asp Lys Ser Val Leu Val Ala
1               5                  10                  15


Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ala Thr Ser Leu Ile Pro
            20                  25                  30


Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro Gly Arg Glu Leu
        35                  40                  45


Ile Tyr Asn Gln Lys Glu Gly His Phe Pro Arg Val Thr Thr Val Ser
    50                  55                  60


Asp Leu Thr Lys Arg Asn Asn Met Asp Phe Ser Ile Arg Ile Gly Asn
65                  70                  75                  80


Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Val Lys Phe Arg Lys
                85                  90                  95


Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala Gly Thr Glu Leu
            100                 105                 110


Ser Val Arg Ala Lys Pro Ser Ala Pro Val Val Ser Gly Pro Ala Ala
        115                 120                 125


Arg Ala Thr Pro Gln His Thr Val Ser Phe Thr Cys Glu Ser His Gly
    130                 135                 140


Phe Ser Pro Arg Asp Ile Thr Leu Lys Trp Phe Lys Asn Gly Asn Glu
145                 150                 155                 160
```

```
Leu Ser Asp Phe Gln Thr Asn Val Asp Pro Val Gly Glu Ser Val Ser
                165                 170                 175

Tyr Ser Ile His Ser Thr Ala Lys Val Val Leu Thr Arg Glu Asp Val
            180                 185                 190

His Ser Gln Val Ile Cys Glu Val Ala His Val Thr Leu Gln Gly Asp
        195                 200                 205

Pro Leu Arg Gly Thr Ala Asn Leu Ser Glu Thr Ile Arg Val Pro Pro
    210                 215                 220

Thr Leu Glu Val Thr Gln Gln Pro Val Arg Ala Glu Asn Gln Val Asn
225                 230                 235                 240

Val Thr Cys Gln Val Arg Lys Phe Tyr Pro Gln Arg Leu Gln Leu Thr
                245                 250                 255

Trp Leu Glu Asn Gly Asn Val Ser Arg Thr Glu Thr Ala Ser Thr Val
            260                 265                 270

Thr Glu Asn Lys Asp Gly Thr Tyr Asn Trp Met Ser Trp Leu Leu Val
        275                 280                 285

Asn Val Ser Ala His Arg Asp Asp Val Lys Leu Thr Cys Gln Val Glu
    290                 295                 300

His Asp Gly Gln Pro Ala Val Ser Lys Ser His Asp Leu Lys Val Ser
305                 310                 315                 320

Ala His Pro Lys Glu Gln Gly Ser Asn Thr Ala Ala Glu Asn Thr Gly
                325                 330                 335

Ser Asn Glu Arg Asn Ile Tyr Ser Lys Tyr Gly Pro Pro Cys Pro Pro
            340                 345                 350

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
        355                 360                 365
```

```
Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr
    370                 375                 380

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
385                 390                 395                 400

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
                405                 410                 415

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
            420                 425                 430

Leu His Gln Asp Trp Leu Ser Gly Lys Glu Tyr Lys Cys Lys Val Ser
        435                 440                 445

Ser Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Asn Ala Thr
    450                 455                 460

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
465                 470                 475                 480

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                485                 490                 495

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
            500                 505                 510

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
        515                 520                 525

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
    530                 535                 540

Asn Val Phe Ser Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr
545                 550                 555                 560

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Ile Glu Gly Arg Met
                565                 570                 575
```

```
Asp His Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu
            580                 585                 590

Asp Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg
        595                 600                 605

Ser Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly
    610                 615                 620

Phe Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn
625                 630                 635                 640

Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His
                645                 650                 655

Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala
            660                 665                 670

Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn
        675                 680                 685

Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala
    690                 695                 700

Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe
705                 710                 715                 720

Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu
                725                 730                 735

Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln
            740                 745                 750

Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val
        755                 760                 765

Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe
    770                 775                 780
```

Thr Ser Phe Gly Leu Leu Lys Leu
785 790

<210> 61
<211> 710
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 61

Glu Glu Glu Leu Gln Val Ile Gln Pro Asp Lys Ser Val Leu Val Ala
1 5 10 15

Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ala Thr Ser Leu Ile Pro
20 25 30

Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro Gly Arg Glu Leu
35 40 45

Ile Tyr Asn Gln Lys Glu Gly His Phe Pro Arg Val Thr Thr Val Ser
50 55 60

Asp Leu Thr Lys Arg Asn Asn Met Asp Phe Ser Ile Arg Ile Gly Asn
65 70 75 80

Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Val Lys Phe Arg Lys
85 90 95

Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala Gly Thr Glu Leu
100 105 110

Ser Val Arg Ala Lys Pro Ser Ala Pro Val Val Ser Gly Pro Ala Ala
115 120 125

Arg Ala Thr Pro Gln His Thr Val Ser Phe Thr Cys Glu Ser His Gly
130 135 140

Phe Ser Pro Arg Asp Ile Thr Leu Lys Trp Phe Lys Asn Gly Asn Glu
145 150 155 160

```
Leu Ser Asp Phe Gln Thr Asn Val Asp Pro Val Gly Glu Ser Val Ser
                165                 170                 175

Tyr Ser Ile His Ser Thr Ala Lys Val Val Leu Thr Arg Glu Asp Val
            180                 185                 190

His Ser Gln Val Ile Cys Glu Val Ala His Val Thr Leu Gln Gly Asp
        195                 200                 205

Pro Leu Arg Gly Thr Ala Asn Leu Ser Glu Thr Ile Arg Val Pro Pro
    210                 215                 220

Thr Leu Glu Val Thr Gln Gln Pro Val Arg Ala Glu Asn Gln Val Asn
225                 230                 235                 240

Val Thr Cys Gln Val Arg Lys Phe Tyr Pro Gln Arg Leu Gln Leu Thr
                245                 250                 255

Trp Leu Glu Asn Gly Asn Val Ser Arg Thr Glu Thr Ala Ser Thr Val
            260                 265                 270

Thr Glu Asn Lys Asp Gly Thr Tyr Asn Trp Met Ser Trp Leu Leu Val
        275                 280                 285

Asn Val Ser Ala His Arg Asp Asp Val Lys Leu Thr Cys Gln Val Glu
    290                 295                 300

His Asp Gly Gln Pro Ala Val Ser Lys Ser His Asp Leu Lys Val Ser
305                 310                 315                 320

Ala His Pro Lys Glu Gln Gly Ser Asn Thr Ala Ala Glu Asn Thr Gly
                325                 330                 335

Ser Asn Glu Arg Asn Ile Tyr Ser Lys Tyr Gly Pro Pro Cys Pro Pro
            340                 345                 350

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
        355                 360                 365
```

```
Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr
    370                 375                 380

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
385                 390                 395                 400

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
                405                 410                 415

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
            420                 425                 430

Leu His Gln Asp Trp Leu Ser Gly Lys Glu Tyr Lys Cys Lys Val Ser
        435                 440                 445

Ser Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Asn Ala Thr
    450                 455                 460

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
465                 470                 475                 480

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                485                 490                 495

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
            500                 505                 510

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
        515                 520                 525

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
    530                 535                 540

Asn Val Phe Ser Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr
545                 550                 555                 560

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Ile Glu Gly Arg Met
                565                 570                 575
```

```
Asp Gln Val Ser His Arg Tyr Pro Arg Ile Gln Ser Ile Lys Val Gln
            580                 585                 590

Phe Thr Glu Tyr Lys Lys Glu Lys Gly Phe Ile Leu Thr Ser Gln Lys
        595                 600                 605

Glu Asp Glu Ile Met Lys Val Gln Asn Asn Ser Val Ile Ile Asn Cys
    610                 615                 620

Asp Gly Phe Tyr Leu Ile Ser Leu Lys Gly Tyr Phe Ser Gln Glu Val
625                 630                 635                 640

Asn Ile Ser Leu His Tyr Gln Lys Asp Glu Glu Pro Leu Phe Gln Leu
                645                 650                 655

Lys Lys Val Arg Ser Val Asn Ser Leu Met Val Ala Ser Leu Thr Tyr
            660                 665                 670

Lys Asp Lys Val Tyr Leu Asn Val Thr Thr Asp Asn Thr Ser Leu Asp
        675                 680                 685

Asp Phe His Val Asn Gly Gly Glu Leu Ile Leu Ile His Gln Asn Pro
    690                 695                 700

Gly Glu Phe Cys Val Leu
705                 710


<210>  62
<211>  182
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Polypeptide

<400>  62

Leu Gln Leu His Trp Arg Leu Gly Glu Met Val Thr Arg Leu Pro Asp
1               5                   10                  15
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Gly Pro Ala Gly Ser Trp Glu Gln Leu Ile Gln Glu Arg Arg Ser His
            20                  25                  30


Glu Val Asn Pro Ala Ala His Leu Thr Gly Ala Asn Ser Ser Leu Thr
        35                  40                  45


Gly Ser Gly Gly Pro Leu Leu Trp Glu Thr Gln Leu Gly Leu Ala Phe
    50                  55                  60


Leu Arg Gly Leu Ser Tyr His Asp Gly Ala Leu Val Val Thr Lys Ala
65                  70                  75                  80


Gly Tyr Tyr Tyr Ile Tyr Ser Lys Val Gln Leu Gly Gly Val Gly Cys
                85                  90                  95


Pro Leu Gly Leu Ala Ser Thr Ile Thr His Gly Leu Tyr Lys Arg Thr
            100                 105                 110


Pro Arg Tyr Pro Glu Glu Leu Glu Leu Leu Val Ser Gln Gln Ser Pro
        115                 120                 125


Cys Gly Arg Ala Thr Ser Ser Ser Arg Val Trp Trp Asp Ser Ser Phe
    130                 135                 140


Leu Gly Gly Val Val His Leu Glu Ala Gly Glu Lys Val Val Val Arg
145                 150                 155                 160


Val Leu Asp Glu Arg Leu Val Arg Leu Arg Asp Gly Thr Arg Ser Tyr
                165                 170                 175


Phe Gly Ala Phe Met Val
            180
```

<210> 63
<211> 759
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 63

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Glu Glu Glu Leu Gln Val Ile Gln Pro Asp Lys Ser Val Leu Val Ala
1               5                  10                  15

Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ala Thr Ser Leu Ile Pro
            20                  25                  30

Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro Gly Arg Glu Leu
        35                  40                  45

Ile Tyr Asn Gln Lys Glu Gly His Phe Pro Arg Val Thr Thr Val Ser
    50                  55                  60

Asp Leu Thr Lys Arg Asn Asn Met Asp Phe Ser Ile Arg Ile Gly Asn
65                  70                  75                  80

Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Val Lys Phe Arg Lys
                85                  90                  95

Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala Gly Thr Glu Leu
            100                 105                 110

Ser Val Arg Ala Lys Pro Ser Ala Pro Val Val Ser Gly Pro Ala Ala
        115                 120                 125

Arg Ala Thr Pro Gln His Thr Val Ser Phe Thr Cys Glu Ser His Gly
    130                 135                 140

Phe Ser Pro Arg Asp Ile Thr Leu Lys Trp Phe Lys Asn Gly Asn Glu
145                 150                 155                 160

Leu Ser Asp Phe Gln Thr Asn Val Asp Pro Val Gly Glu Ser Val Ser
                165                 170                 175

Tyr Ser Ile His Ser Thr Ala Lys Val Val Leu Thr Arg Glu Asp Val
            180                 185                 190

His Ser Gln Val Ile Cys Glu Val Ala His Val Thr Leu Gln Gly Asp
        195                 200                 205
```

```
Pro Leu Arg Gly Thr Ala Asn Leu Ser Glu Thr Ile Arg Val Pro Pro
    210                 215                 220

Thr Leu Glu Val Thr Gln Gln Pro Val Arg Ala Glu Asn Gln Val Asn
225                 230                 235                 240

Val Thr Cys Gln Val Arg Lys Phe Tyr Pro Gln Arg Leu Gln Leu Thr
                245                 250                 255

Trp Leu Glu Asn Gly Asn Val Ser Arg Thr Glu Thr Ala Ser Thr Val
            260                 265                 270

Thr Glu Asn Lys Asp Gly Thr Tyr Asn Trp Met Ser Trp Leu Leu Val
        275                 280                 285

Asn Val Ser Ala His Arg Asp Asp Val Lys Leu Thr Cys Gln Val Glu
    290                 295                 300

His Asp Gly Gln Pro Ala Val Ser Lys Ser His Asp Leu Lys Val Ser
305                 310                 315                 320

Ala His Pro Lys Glu Gln Gly Ser Asn Thr Ala Ala Glu Asn Thr Gly
                325                 330                 335

Ser Asn Glu Arg Asn Ile Tyr Ser Lys Tyr Gly Pro Pro Cys Pro Pro
            340                 345                 350

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
        355                 360                 365

Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg Thr Pro Glu Val Thr
    370                 375                 380

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
385                 390                 395                 400

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
                405                 410                 415
```

```
Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
            420                 425                 430

Leu His Gln Asp Trp Leu Ser Gly Lys Glu Tyr Lys Cys Lys Val Ser
        435                 440                 445

Ser Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Asn Ala Thr
    450                 455                 460

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
465                 470                 475                 480

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                485                 490                 495

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
            500                 505                 510

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
        515                 520                 525

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
    530                 535                 540

Asn Val Phe Ser Cys Ser Val Leu His Glu Ala Leu His Asn His Tyr
545                 550                 555                 560

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Ile Glu Gly Arg Met
                565                 570                 575

Asp Leu Gln Leu His Trp Arg Leu Gly Glu Met Val Thr Arg Leu Pro
            580                 585                 590

Asp Gly Pro Ala Gly Ser Trp Glu Gln Leu Ile Gln Glu Arg Arg Ser
        595                 600                 605

His Glu Val Asn Pro Ala Ala His Leu Thr Gly Ala Asn Ser Ser Leu
    610                 615                 620
```

```
Thr Gly Ser Gly Gly Pro Leu Leu Trp Glu Thr Gln Leu Gly Leu Ala
625                 630                 635                 640

Phe Leu Arg Gly Leu Ser Tyr His Asp Gly Ala Leu Val Val Thr Lys
                645                 650                 655

Ala Gly Tyr Tyr Tyr Ile Tyr Ser Lys Val Gln Leu Gly Gly Val Gly
            660                 665                 670

Cys Pro Leu Gly Leu Ala Ser Thr Ile Thr His Gly Leu Tyr Lys Arg
        675                 680                 685

Thr Pro Arg Tyr Pro Glu Glu Leu Glu Leu Leu Val Ser Gln Gln Ser
    690                 695                 700

Pro Cys Gly Arg Ala Thr Ser Ser Ser Arg Val Trp Trp Asp Ser Ser
705                 710                 715                 720

Phe Leu Gly Gly Val Val His Leu Glu Ala Gly Glu Lys Val Val Val
                725                 730                 735

Arg Val Leu Asp Glu Arg Leu Val Arg Leu Arg Asp Gly Thr Arg Ser
            740                 745                 750

Tyr Phe Gly Ala Phe Met Val
        755
```