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(54) **Title**: SIRP ALPHA-ANTIBODY FUSION PROTEINS

(57) **Abstract**: SIRPabodies comprise an immunoglobulin variable region, which may specifically bind a tumor antigen, viral antigen, etc., fused to a sequence comprising a binding domain of SIRPα. The binding domain of SIRPα comprises at least the N-terminal Ig-like domain of SIRPα, and may further comprise additional SIRPα sequences. The SIRPabodies find use in therapeutic methods that benefit from the combined activity of blocking CD47 activity, and antibody targeting, e.g. in the treatment of cancer, etc. In some specific embodiments, the SIRPabody comprises anti-CD20 activity and a SIRP binding domain; anti-CD99 and a SIRP binding domain; or anti-TIM3 activity and a SIRP α binding domain.

SIRP ALPHA-ANTIBODY FUSION PROTEINS

BACKGROUND OF THE INVENTION

[0001] Macrophages clear pathogens and damaged or aged cells from the blood stream via phagocytosis. Cell-surface CD47 interacts with its receptor on macrophages, SIRPα, to inhibit phagocytosis of normal, healthy cells. CD47 is a broadly expressed transmembrane glycoprotein with a single Ig-like domain and five membrane spanning regions, which functions as a cellular ligand for SIRPα with binding mediated through the NH_2-terminal V-like domain of SIRPα. SIRPα is expressed primarily on myeloid cells, including macrophages, granulocytes, myeloid dendritic cells (DCs), mast cells, and their precursors, including hematopoietic stem cells.

[0002] SIRPα inhibits the phagocytosis of host cells by macrophages, where the ligation of SIRPα on macrophages by CD47 expressed on the host target cell generates an inhibitory signal mediated by SHP-1 that negatively regulates phagocytosis. SIRPα acts to detect signals provided by "self," to negatively control innate immune effector function against these cells.

[0003] In keeping with the role of CD47 to inhibit phagocytosis of normal cells, there is evidence that it is transiently upregulated on hematopoietic stem cells (HSCs) and progenitors just prior to and during their migratory phase, and that the level of CD47 on these cells determines the probability that they are engulfed in vivo.

[0004] The present invention provides immunoglobulin fusion proteins that inhibit the interaction of CD47 with SIRP leading to phagocytosis as a result of disrupting the negative regulatory signal.

SUMMARY OF THE INVENTION

[0005] Compositions and methods are provided relating to fusion proteins, herein termed SIRPabodies, that comprise an immunoglobulin variable region, which may specifically bind a tumor antigen, viral antigen, etc., fused to a sequence comprising a binding domain of SIRPα. The binding domain of SIRPα comprises at least the N-terminal Ig-like domain of SIRPα, and may further comprise additional SIRPα sequences. The SIRPabodies find use in therapeutic methods that benefit from the combined activity of blocking CD47 activity, and antibody targeting, e.g. in the treatment of cancer, etc. In some specific embodiments, the SIRPabody comprises an anti-CD20 binding domain and a SIRPα binding domain. In other specific embodiments the SIRPabody comprises an anti-CD99 binding domain and a SIRPα binding domain. In other specific embodiments the SIRPabody comprises an anti-TIM3 binding domain and a SIRPα binding domain.

[0006] SIRPabody polypeptide molecules of the invention comprise two functional domains: an immunoglobulin variable region domain, and the N-terminal Ig-like domain of SIRPα, for

example as shown in SEQ ID NO:1 and variants thereof, including without limitation allelic polymorphisms. In some embodiments the SIRPabody comprises a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of an immunoglobulin (V_L) specific for a first epitope; and a second polypeptide comprising (i) a first domain comprising a binding region of a heavy chain variable region domain of an immunoglobulin (V_H) specific for a first epitope; and (ii) a second domain comprising the N-terminal Ig-like domain of SIRPα. In some embodiments, the first polypeptide and the second polypeptide further comprise the respective heavy and light chain constant region domains of the immunoglobulin, i.e. C_L and C_H. In some embodiments, the second polypeptide comprises the N-terminal Ig-like domain of SIRPα fused to the amino terminus of the V_H domain (N-terminal SIRPabody, or NH-SIRPabody). In other embodiments the second polypeptide comprises the N-terminal Ig-like domain of SIRPα fused to the amino terminus of the C_H domains (C-terminal SIRPabody, or CH-SIRPabody).

[0007] In an alternative embodiment, the SIRPabody comprises a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of an immunoglobulin (V_L) specific for a first epitope; and (ii) a second domain comprising the N-terminal Ig-like domain of SIRPα; and a second polypeptide comprising (i) a first domain comprising a binding region of a heavy chain variable region domain of an immunoglobulin (V_H) specific for a first epitope. In some embodiments, the first polypeptide and the second polypeptide further comprise the respective heavy and light chain constant region domains of the immunoglobulin, i.e. C_L and C_H. In some embodiments, the first polypeptide comprises the N-terminal Ig-like domain of SIRPα fused to the amino terminus of the V_L domain (NL-SIRPabody). In other embodiments the second polypeptide comprises the N-terminal Ig-like domain of SIRPα fused to the amino terminus of the C_L domains (CL-SIRPabody).

[0008] The SIRPα domain and the V_H or C_H domains may be separated by a short linker. The peptide linker may be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids in length, and is of sufficient length and amino acid composition to minimize steric hindrance between the binding domains. In some embodiments the linker is glycine and/or serine; exemplary is one or more subunits of SEQ ID NO:2. Alternatively the sequence of the constant region can provide the linker, for example as shown in SEQ ID NO:3.

[0009] The SIRPabodies of the invention are particularly efficacious in the treatment of disease, e.g. increasing phagocytosis of living CD47 expressing cells. Treatment may be systemic or localized, e.g. delivery by intratumoral injection, *etc.* In certain embodiments, methods are provided for a targeted cytotoxicity therapy that (a) blocks CD47 activity through the SIRPα binding domain and may additionally (b) provide antibody mediated targeting

and/or cell killing as a result of binding to the immunoglobulin variable region domain. A targeted cell population suspected of expressing on the cell surface the cognate antigen for the immunoglobulin variable region domain of a SIRPabody is contacted with an effective dose of the SIRPabody, where the dose is sufficient to increase killing of the targeted cells, relative to the level of killing obtained with the immunoglobulin alone. In such embodiments, the F_C region of the immunoglobulin is generally present, and the activity may be compared to the activity of the intact immunoglobulin.

[0010] In some such embodiments the cognate antigen is CD20. Antibodies that specifically target CD20 are well known in the art, including human and humanized antibodies available for therapeutic purposes, e.g. Tositumumab, Rituximab, Ibritumomab Tiuxetan, Veltuzumab, AME-133v, Ofatumumab, R7159, *etc.* The targeted cells in such an embodiment are CD20 positive cancer cells. The contacting may be performed *in vivo*, and includes the treatment of malignancies, including without limitation B cell non-Hodgkin lymphomas, hairy cell leukemia, B-cell chronic lymphocytic leukemia, melanoma cancer stem cells, etc. In some embodiments, treatment of an individual with such a malignancy comprises the steps of administering an effective dose of a SIRPabody that causes increased killing of CD20+ malignant cells, i.e. to increase by greater than about 20%, to increase by greater than about 30%, to increase by greater than about 40%, to increase by greater than about 50%, to increase by greater than about 75%, to increase by greater than about 90%, to increase by greater than about 95%, to increase by greater than about 99% or more relative to treatment with the corresponding immunoglobulin lacking the SIRPα binding domain. A synergistic response may be obtained, where, for example, the reduction in tumor cell population with the SIRPabody is greater than the reduction obtained with one or both of soluble SIRPα binding domain and anti-CD20 immunoglobulin. SIRPabodies for this purpose include without limitation CD20-2GL-SIRPα, CD20-4GL-SIRPα, and SIRPα-CD20 provided herein.

[0011] In other embodiments the cognate antigen is CD99. Antibodies specific for CD99 and are known in the art and commercially available, e.g. 12E7, HCD99, F21, O13, etc. CD99 is expressed on a number of cancers, including without limitation Ewing's sarcoma tumors, thymic tumors, synovial sarcoma, haemangiopericytoma, and meningioma, small cell lung cancer, AML, diffuse large B-cell lymphoma (DLBCL), etc. In some embodiments, treatment of an individual with such a malignancy comprises the steps of administering an effective dose of a SIRPabody that causes increased killing of CD99+ malignant cells, i.e. to increase by greater than about 20%, to increase by greater than about 30%, to increase by greater than about 40%, to increase by greater than about 50%, to increase by greater than about 75%, to increase by greater than about 90%, to increase by greater than about 95%, to increase by greater than about 99% or more relative to treatment with the corresponding immunoglobulin lacking the SIRPα binding domain. A synergistic response may be obtained, where, for

example, the reduction in tumor cell population with the SIRPabody is greater than the reduction obtained with one or both of soluble SIRPα binding domain and anti-CD99 immunoglobulin.

[0012] In other embodiments the cognate antigen is TIM3. Antibodies specific for TIM3 and are known in the art and commercially available, e.g. 1F38-2E2, etc. CD99 is expressed on cetain cancer cells, including without limitation AML. In some embodiments, treatment of an individual with such a malignancy comprises the steps of administering an effective dose of a SIRPabody that causes increased killing of TIM3+ malignant cells, i.e. to increase by greater than about 20%, to increase by greater than about 30%, to increase by greater than about 40%, to increase by greater than about 50%, to increase by greater than about 75%, to increase by greater than about 90%, to increase by greater than about 95%, to increase by greater than about 99% or more relative to treatment with the corresponding immunoglobulin lacking the SIRPα binding domain. A synergistic response may be obtained, where, for example, the reduction in tumor cell population with the SIRPabody is greater than the reduction obtained with one or both of soluble SIRPα binding domain and anti-TIM3 immunoglobulin.

[0013] In other related embodiments, an anti-CD20 SIRPabody is used in a method of treating autoimmune diseases with a B cell component, including without limitation rheumatoid arthritis, multiple sclerosis, Type I diabetes, Type II diabetes, systemic lupus erythematosus, and the like. In such methods targeted cytotoxicity (a) blocks CD47 activity through the SIRPα binding domain and (b) provides antibody mediated cell killing through the immunoglobulin variable region domain. A targeted B cell population expressing CD20 is contacted with an effective dose of the SIRPabody, where the dose is sufficient to increase killing of the targeted cells, relative to the level of killing obtained with the immunoglobulin alone. In such embodiments, the F_C region of the immunoglobulin is generally present, and the activity may be compared to the activity of the intact immunoglobulin.

[0014] In yet another embodiment of the present invention, the SIRPabodies of the invention can be used to treat a variety of diseases and disorders. Accordingly, the present invention is directed to a method for treating a disease or disorder comprising administering to a patient in need thereof an effective amount of a SIRPabody of the invention, in which the SIRPabody has been selected to provide a specific level of targeting correlated with the binding specificity of the immunoglobulin, coupled with blocking CD47 activity. Such methods include those contemplated by US Patent no. 8,562,997, international applications US2014/014905 and US2014/035167, each herein specifically incorporated by reference. In further such embodiments of the invention, the antigen is a tumor antigen.

[0015] The immunoglobulin portion of the SIRPabody may comprise constant region sequences that are characteristic of mouse, rabbit, primate, human, *etc.*, antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, *etc.*, as is known in the art. Moreover, the term "antibody" as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, embodiments, an immunoglobulin portion of the SIRPabody utilized in accordance with the present invention is in a format selected from, but not limited to, IgG, IgE and IgM, bi- or multi- specific antibodies (e.g., Zybodies®, etc), single chain Fvs, polypeptide-Fc fusions, Fabs, cameloid antibodies, masked antibodies (e.g., Probodies®), Small Modular ImmunoPharmaceuticals ("SMIPs™"), single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, minibodies, BiTE®s, ankyrin repeat proteins or DARPINs®, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Fynomers®, Centyrins®, and a KALBITOR®.

[0016] For example, the SIRPabody may comprise a full length chimeric or humanized antibody, *e.g.* having a human immunoglobulin constant region of any isotype, *e.g.* IgG1, IgG2a, IgG2b, IgG3, IgG4, IgA, *etc.* or an antibody fragment, e.g. a $F(ab')_2$ fragment, and F(ab) fragment, *etc.* Furthermore, the SIRPabody may be labeled with a detectable label, immobilized on a solid phase and/or conjugated with a heterologous compound.

[0017] The invention further provides: isolated nucleic acid encoding the SIRPabodies and variants thereof; a vector comprising that nucleic acid, optionally operably linked to control sequences recognized by a host cell transformed with the vector; a host cell comprising that vector; a process for producing the antibody comprising culturing the host cell so that the nucleic acid is expressed and, optionally, recovering the antibody from the host cell culture (e.g. from the host cell culture medium). The invention also provides a composition comprising one or more SIRPabodies and a pharmaceutically acceptable carrier or diluent. This composition for therapeutic use is sterile and may be lyophilized, *e.g.* being provided as a pre-pack in a unit dose with diluent and delivery device, *e.g.* inhaler, syringe, *etc.*

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1: Schematic of SIRPα-rituximab reagents (SIRPabodies). The N-terminal immunoglobulin fold from wild type SIRPα (green) was engineered onto either the N or C terminus of the heavy chain of rituximab (clone 2B8). The light chain sequence is provided as SEQ ID NO:4, where the signal sequence is amino acid residues 1-22. A polyglycine serine linker SEQ ID NO:2, $(GGGGS)_2$ or $(GGGGS)_4$ was used to fuse SIRPα onto the C terminus of the heavy chain to create CD20-2GL-SIRPα (SEQ ID NO:6) or CD20-4GL-SIRPα (SEQ ID

NO:10), respectively. The signal sequence is amino acid residues 1-19, the linker is residues 471-480 or 471-490, respectively. The SIRPα sequence is residues 481-599, or 490-609, respectively. A linker derived from the N terminal amino acids of the CH1 domain SEQ ID NO:3, (ASTKGPSVFPLAP) was used to fuse SIRPα onto the N terminus of the heavy chain, shoan as SEQ ID NO:8, where the signal sequence is amino acid residues 1-17, the SIRPα sequence is residues 18-136, the linker is residues 137-149.

[0019] Figure 2: Construction of SIRPα-rituximab bispecific reagents. SDS-PAGE analysis of the indicated purified antibodies under non-reducing (left) and reducing (right) conditions. Anti-CD20 antibody was included as a reference for the sizes of the parental heavy and light chains.

[0020] Figure 3: SIRPα-rituximab bispecific reagents bind to CD20 on the cell surface. The indicated antibodies were used to stain rat YB2/0 cells engineered to express human CD20, but not human CD47 (CD20+CD47-) prior to detection with anti-human secondary antibody by flow cytometry. 2B8 = anti-CD20, B6H12 = anti-CD47.

[0021] Figure 4: SIRPα-rituximab bispecific reagents bind to CD20 with similar affinity as parental antibody. CD20+CD47- YB2/0 cells were incubated with the indicated primary antibodies over a range of concentrations prior to staining with 10 μg/ml DyLight 488anti-CD20 and detection by flow cytometry. Mean fluorescence intensity (MFI) of the DyLight 488 signal for each condition was measured by flow cytometry.

[0022] Figure 5: SIRPα-rituximab bispecific reagents bind to CD47 on the cell surface with reduced affinity relative to anti-CD47. The indicated antibodies were used to stain rat YB2/0 cells engineered to express human CD47, but not human CD20 (CD20-CD47+) prior to detection with fluorescently tagged anti-human secondary antibody by flow cytometry. 2B8 = anti-CD20, B6H12 = anti-CD47.

[0023] Figure 6: Binding of SIRPα-rituximab bispecific reagents to CD47 is outcompeted by labeled anti-CD47. CD20-CD47+ YB2/0 cells were incubated with the indicated primary antibodies over a range of concentrations prior to staining with 10 μg/ml APC anti-CD47 and detection by flow cytometry. MFI of the APC signal for each condition is reported.

[0024] Figure 7: SIRPα-rituximab bispecific reagents bind to CD47 with reduced affinity relative to anti-CD47 and other bispecific antibody formats. Binding of the indicated antibodies to the recombinant CD47 antigen was measured by ELISA over a range of concentrations. Immobilized human CD47 tagged with mouse Fc was used to capture the indicated antibodies prior to detection with HRP-conjugated antibody directed against the human kappa light chain. Data are representative of three experiments performed in triplicate.

[0025] Figure 8: SIRPα-rituximab bispecific reagents have reduced affinity to CD47 relative to anti-CD47. Kinetic association and dissociation parameters, along with calculated affinity (Kd)

were measured by surface plasmon resonance using Biacore. The surface was coated with the indicated antibodies via amine-coupling prior to exposure to CD47-His analyte.

[0026] Figure 9: SIRPα-rituximab bispecific reagents bind to CD20 and CD47 simultaneously. Schematic of assay to determine simultaneous binding to CD20 and CD47. Double positive events indicate simultaneous binding to CD20 and CD47 by the indicated antibodies.

[0027] Figure 10: SIRPα-rituximab bispecific reagents bind to CD20 and CD47 on dual antigen Raji cells. CD20+CD47+ Raji cells were incubated with the indicated primary antibodies at 10 μg/ml prior to staining with APC anti-CD47 and detection by flow cytometry. CD20+CD47+ Raji cells were incubated with the indicated primary antibodies at 10 μg/ml prior to staining with DyLight 488anti-CD20 and detection by flow cytometry.

[0028] Figure 11: SIRPα-rituximab bispecific reagents preferentially bind to dual antigen tumor cells in the presence of excess CD47-only expressing red blood cells. (A) Schematic of the experimental design to assay for selectivity in binding to dual antigen-expressing cells in the presence of an excess of CD47-only expressing cells. GFP labeled CD20+CD47+ cells were mixed with a 10-fold excess of CD20-CD47+ human red blood cells (RBCs). Cell mixtures were incubated with primary antibody prior to staining with PE anti-human Fcγ secondary and 10 μg/ml APC anti-CD47, and analyzed by flow cytometry. (B,C) Tumor cells were distinguished from RBCs on the basis of GFP expression. The indicated primary antibodies were used at 10ug/ml and binding to cells was detected with secondary antibody staining (PE anti-human Fc). 2B8 = anti-CD20, B6H12 = anti-CD47. (D) Binding of APC anti-CD47 to cells is reported as MFI normalized to isotype control.

[0029] Figure 12: SIRPα-rituximab bispecific reagents induce phagocytosis of dual antigen cells. Phagocytosis of CD20+CD47+ Raji-GFP cells by human macrophages was assessed by flow cytometry. The percentage of GFP+ macrophages was normalized to the maximal response for each donor. Data averaged from 3 independent donors and are ±SD.

[0030] Figure 13: CD20-2GL-SIRPα eliminates lymphoma *in vivo.* (A) NSG mice transplanted subcutaneously with luciferase-expressing Raji cells were treated with daily injections of 200 μg mouse IgG control, anti-CD47, SIRPα-Fc, rituximab, CD20-2GL-SIRPα, or 200 μg anti-CD47 + 200 μg rituximab. (n = 5 per treatment group). Luciferase imaging was averaged for all mice in each treatment group. Arrows indicate start (day 7) and stop (day 20) of treatment. Rituximab treatment was compared to CD20-2GL-SIRPα at day 42 (*$p<0.05$ by t test). (B) Kaplan-Meier survival analysis with p-values calculated comparing rituximab single antibody treatment to combination treatment/CD20-2GL-SIRPα. (*$p<0.05$ by log-rank Mantel-Cox test).

[0031] Figure 14. CD20-2GL-SIRPα HC Reduces Lymphoma Burden and Extends Survival In Vivo. (A) NSG mice were transplanted subcutaneously with Raji-luciferase cells. Seven days later, mice were treated with 14 daily doses of 200 μg IgG (n = 15), SIRPα-Fc (n = 15), rituximab (n = 15), CD20-2GL-SIRPα HC (n = 15) or 200 μg SIRPα-Fc + 200 μg rituximab (n =

10). Expansion of Raji-luciferase cells was evaluated by bioluminescence imaging. Each point represents a measurement from an independent mouse and lines indicate median values for each treatment group. p values were derived by t test and compare rituximab to CD20-2GL-SIRPα HC for each time point. (B) Kaplan-Meier survival analysis was performed. Arrows indicated start (day 7) and stop (day 21) of treatment. Statistical analysis was performed by Mantel-Cox and compares rituximab to CD20-2GL-SIRPα HC. (C) NSG mice were transplanted intravenously with Raji-luciferase cells. Four days later, mice were administered 21 daily doses of antibody treatment as described in (A). Each point represents a measurement from an independent mouse (n=10). Lines indicate mean values for each treatment group. p values were derived by t test and compare rituximab to CD20-2GL-SIRPα HC for each time point. (D) Kaplan-Meier survival analysis was performed. Arrows indicated start (day 4) and stop (day 25) of treatment. Statistical analysis was performed by Mantel-Cox and compares rituximab to CD20-2GL-SIRPα HC.

DETAILED DESCRIPTION OF THE INVENTION

[0032] SIRPabodies comprise an immunoglobulin variable region, which may specifically bind a tumor antigen, viral antigen, etc., fused to a sequence comprising a binding domain of SIRPα. The binding domain of SIRPα comprises at least the N-terminal Ig-like domain of SIRPα, and may further comprise additional SIRPα sequences. The SIRPabodies find use in therapeutic methods that benefit from the combined activity of blocking CD47 activity, and antibody targeting, e.g. in the treatment of cancer, etc. In some specific embodiments, the SIRPabody comprises anti-CD20 activity and a SIRPα binding domain.

Definitions

[0033] In the description that follows, a number of terms conventionally used in the field of cell culture are utilized. In order to provide a clear and consistent understanding of the specification and claims, and the scope to be given to such terms, the following definitions are provided.

[0034] The terms "CD47 binding domain agents", with respect to the interaction between SIRPα and its ligand CD47 refer to molecules that prevent the binding of SIRPα and CD47. For development purposes the binding may be performed under experimental conditions, *e.g.* using isolated proteins as binding partners, using portions of proteins as binding partners, using yeast display of proteins or portions of proteins as binding partners, and the like.

[0035] For therapeutic purposes the binding of SIRPα and CD47 is typically an event between two cells, where each cell expresses one of the binding partners. Of particular interest is the expression of SIRPα on phagocytic cells, such as macrophages; and the expression of CD47 on cells that may be targets for phagocytosis, e.g. tumor cells, circulating

hematopoietic cells, and the like. Inhibitors may be identified using *in vitro* and *in vivo* assays for receptor or ligand binding or signaling.

[0036] For the purposes of the invention a blocking agent comprises a SIRPα binding domain, for example the N-terminal immunoglobulin fold domain of SEQ ID NO:1, and variants, including without limitation allelic variants. Relative to the human native SIRPα sequences, (for example see Genbank accession no. AAH75849, and variants NM_080792.2; XM_005260670.1; XM_005260669.1; NM_001040023.1; NM_001040022.1, herein specifically incorporated by reference) a soluble SIRPα binding domain may comprise the d1 domain of SIRPα, corresponding to residues 31 to 149 of the native full-length human protein. In such embodiments, the soluble SIRPα binding domain may consist of all or a portion of the d1 domain; may further comprise one or more amino acids from SIRPα outside of the d1 domain.

[0037] SIRPα binding domains may be at least about 100 amino acids in length, at least about 110, at least about 120, at least about 150, at least about 200 amino acids in length, up to the full-length of the wild-type protein at the transmembrane domain, i.e. about 343 amino acids in length, and is optionally fused to a heterologous polypeptide.

[0038] A low affinity SIRPα sequence is generally preferred for the purposes of the invention, i.e. an affinity equivalent to the affinity of the native protein for binding with CD47. However, in some embodiments an increased affinity may be preferred, for which purpose the variants set forth in, for example, WO 2013/109752 (herein specifically incorporated by reference) may be used.

[0039] The SIRPα sequence may be a variant of the native human sequence. As used herein, the term "variant" refers to an entity that shows significant structural identity with a reference entity but differs structurally from the reference entity in the presence or level of one or more chemical moieties as compared with the reference entity. In many embodiments, a variant also differs functionally from its reference entity. In general, whether a particular entity is properly considered to be a "variant" of a reference entity is based on its degree of structural identity with the reference entity. As will be appreciated by those skilled in the art, any biological or chemical reference entity has certain characteristic structural elements. A variant, by definition, is a distinct chemical entity that shares one or more such characteristic structural elements. A polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular biological function. For example, a variant polypeptide may differ from a reference polypeptide as a result of one or more differences in amino acid sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, etc) covalently attached to the polypeptide backbone. In some embodiments, a variant polypeptide shows an overall sequence identity with a reference

polypeptide that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. Alternatively or additionally, in some embodiments, a variant polypeptide does not share at least one characteristic sequence element with a reference polypeptide. In some embodiments, the reference polypeptide has one or more biological activities. In some embodiments, a variant polypeptide shares one or more of the biological activities of the reference polypeptide. In some embodiments, a variant polypeptide lacks one or more of the biological activities of the reference polypeptide. In some embodiments, a variant polypeptide shows a reduced level of one or more biological activities as compared with the reference polypeptide. In many embodiments, a polypeptide of interest is considered to be a "variant" of a parent or reference polypeptide if the polypeptide of interest has an amino acid sequence that is identical to that of the parent but for a small number of sequence alterations at particular positions. Typically, fewer than 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% of the residues in the variant are substituted as compared with the parent. In some embodiments, a variant has 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substituted residue as compared with a parent. Often, a variant has a very small number (e.g., fewer than 5, 4, 3, 2, or 1) number of substituted functional residues (i.e., residues that participate in a particular biological activity). Furthermore, a variant typically has not more than 5, 4, 3, 2, or 1 additions or deletions, and often has no additions or deletions, as compared with the parent. Moreover, any additions or deletions are typically fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly are fewer than about 5, about 4, about 3, or about 2 residues. In some embodiments, the parent or reference polypeptide is one found in nature. As will be understood by those of ordinary skill in the art, a plurality of variants of a particular polypeptide of interest may commonly be found in nature, particularly when the polypeptide of interest is an infectious agent polypeptide.

[0040] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0041] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an .alpha. carbon

that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0042] The terms "subject," "individual," and "patient" are used interchangeably herein to refer to a mammal being assessed for treatment and/or being treated. In an embodiment, the mammal is a human. The terms "subject," "individual," and "patient" encompass, without limitation, individuals having cancer. Subjects may be human, but also include other mammals, particularly those mammals useful as laboratory models for human disease, *e.g.* mouse, rat, *etc.* Also included are mammals such as domestic and other species of canines, felines, and the like.

[0043] The terms "cancer," "neoplasm," and "tumor" are used interchangeably herein to refer to cells which exhibit autonomous, unregulated growth, such that they exhibit an aberrant growth phenotype characterized by a significant loss of control over cell proliferation. Cells of interest for detection, analysis, or treatment in the present application include precancerous (*e.g.*, benign), malignant, pre-metastatic, metastatic, and non-metastatic cells. Cancers of virtually every tissue are known. The phrase "cancer burden" refers to the quantum of cancer cells or cancer volume in a subject. Reducing cancer burden accordingly refers to reducing the number of cancer cells or the cancer volume in a subject. The term "cancer cell" as used herein refers to any cell that is a cancer cell or is derived from a cancer cell e.g. clone of a cancer cell. Many types of cancers are known to those of skill in the art, including solid tumors such as carcinomas, sarcomas, glioblastomas, melanomas, lymphomas, myelomas, etc., and circulating cancers such as leukemias. Examples of cancer include but are not limited to, ovarian cancer, breast cancer, colon cancer, lung cancer, prostate cancer, hepatocellular cancer, gastric cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, thyroid cancer, renal cancer, carcinoma, melanoma, head and neck cancer, and brain cancer.

[0044] The "pathology" of cancer includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of inflammatory or immunological response, neoplasia, premalignancy, malignancy, invasion of surrounding or distant tissues or organs, such as lymph nodes, *etc.*

[0045] As used herein, the terms "cancer recurrence" and "tumor recurrence," and grammatical variants thereof, refer to further growth of neoplastic or cancerous cells after

diagnosis of cancer. Particularly, recurrence may occur when further cancerous cell growth occurs in the cancerous tissue. "Tumor spread," similarly, occurs when the cells of a tumor disseminate into local or distant tissues and organs; therefore tumor spread encompasses tumor metastasis. "Tumor invasion" occurs when the tumor growth spread out locally to compromise the function of involved tissues by compression, destruction, or prevention of normal organ function.

[0046] As used herein, the term "metastasis" refers to the growth of a cancerous tumor in an organ or body part, which is not directly connected to the organ of the original cancerous tumor. Metastasis will be understood to include micrometastasis, which is the presence of an undetectable amount of cancerous cells in an organ or body part which is not directly connected to the organ of the original cancerous tumor. Metastasis can also be defined as several steps of a process, such as the departure of cancer cells from an original tumor site, and migration and/or invasion of cancer cells to other parts of the body.

[0047] Non-Hodgkin lymphomas (NHL) are a heterogeneous group of disorders involving malignant monoclonal proliferation of lymphoid cells in lymphoreticular sites, including lymph nodes, bone marrow, the spleen, the liver, and the gastrointestinal tract. Presenting symptoms usually include peripheral lymphadenopathy. Compared with Hodgkin lymphoma, there is a greater likelihood of disseminated disease at the time of diagnosis. However, NHL is not one disease but rather a category of lymphocyte malignancies. These types can be divided into aggressive (fast-growing) and indolent (slow-growing) types, and they can be formed from either B-cells or T-cells. B-cell non-Hodgkin lymphomas include Burkitt lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma, among others. T-cell non-Hodgkin lymphomas include mycosis fungoides, anaplastic large cell lymphoma, and precursor T-lymphoblastic lymphoma. Lymphomas that occur after bone marrow or stem cell transplantation are usually B-cell non-Hodgkin lymphomas. Prognosis and treatment depend on the stage and type of disease.

[0048] Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), accounting for approximately 30% of all newly diagnosed cases and more than 80% of aggressive lymphomas. Recent insights into the pathogenesis of DLBCL suggest that it is a heterogeneous group of B-cell lymphomas rather than a single clinicopathologic entity. Multiple histologic subtypes and morphologic variants are recognized, a variety of molecular and genetic abnormalities are variably present, and patients exhibit a wide range of clinical presentations and outcomes. Gene-expression profiling studies have identified at least 3 distinct molecular subtypes of DLBCL, one with an expression profile similar to normal germinal center B cells (GCB subtype), one mimicking activated peripheral-blood B cells (ABC

subtype), and a third, primary mediastinal large B-cell lymphoma (PMBCL), typically presenting with mediastinal lymphadenopathy and displaying some molecular genetic similarities to Hodgkin lymphoma. A small number of cases do not fit into any of these categories and have been designated as "unclassifiable."

[0049] The term “sample” with respect to a patient encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents; washed; or enrichment for certain cell populations, such as cancer cells. The definition also includes sample that have been enriched for particular types of molecules, *e.g.*, nucleic acids, polypeptides, *etc.* The term “biological sample” encompasses a clinical sample, and also includes tissue obtained by surgical resection, tissue obtained by biopsy, cells in culture, cell supernatants, cell lysates, tissue samples, organs, bone marrow, blood, plasma, serum, and the like. A “biological sample” includes a sample obtained from a patient’s cancer cell, *e.g.*, a sample comprising polynucleotides and/or polypeptides that is obtained from a patient’s cancer cell (*e.g.*, a cell lysate or other cell extract comprising polynucleotides and/or polypeptides); and a sample comprising cancer cells from a patient. A biological sample comprising a cancer cell from a patient can also include non-cancerous cells.

[0050] The term “diagnosis” is used herein to refer to the identification of a molecular or pathological state, disease or condition, such as the identification of a molecular subtype of breast cancer, prostate cancer, or other type of cancer.

[0051] The term “prognosis” is used herein to refer to the prediction of the likelihood of cancer-attributable death or progression, including recurrence, metastatic spread, and drug resistance, of a neoplastic disease, such as ovarian cancer. The term “prediction” is used herein to refer to the act of foretelling or estimating, based on observation, experience, or scientific reasoning. In one example, a physician may predict the likelihood that a patient will survive, following surgical removal of a primary tumor and/or chemotherapy for a certain period of time without cancer recurrence.

[0052] As used herein, the terms “treatment,” “treating,” and the like, refer to administering an agent, or carrying out a procedure, for the purposes of obtaining an effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of effecting a partial or complete cure for a disease and/or symptoms of the disease. “Treatment,” as used herein, may include treatment of a tumor in a mammal, particularly in a human, and includes: (a) preventing the disease or a symptom of a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it (*e.g.*, including diseases that may be associated with or caused

by a primary disease; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease, *i.e.*, causing regression of the disease.

[0053] Treating may refer to any indicia of success in the treatment or amelioration or prevention of an cancer, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the disease condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the compounds or agents of the present invention to prevent or delay, to alleviate, or to arrest or inhibit development of the symptoms or conditions associated with ocular disease. The term "therapeutic effect" refers to the reduction, elimination, or prevention of the disease, symptoms of the disease, or side effects of the disease in the subject.

[0054] "In combination with", "combination therapy" and "combination products" refer, in certain embodiments, to the concurrent administration to a patient of a first therapeutic and the compounds as used herein. When administered in combination, each component can be administered at the same time or sequentially in any order at different points in time. Thus, each component can be administered separately but sufficiently closely in time so as to provide the desired therapeutic effect.

[0055] In some embodiments, treatment is accomplished by administering a SIRPabody in combination with a cytotoxic agent. One exemplary class of cytotoxic agents are chemotherapeutic agents. Exemplary chemotherapeutic agents include, but are not limited to, aldesleukin, altretamine, amifostine, asparaginase, bleomycin, capecitabine, carboplatin, carmustine, cladribine, cisapride, cisplatin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, docetaxel, doxorubicin, dronabinol, duocarmycin, epoetin alpha, etoposide, filgrastim, fludarabine, fluorouracil, gemcitabine, granisetron, hydroxyurea, idarubicin, ifosfamide, interferon alpha, irinotecan, lansoprazole, levamisole, leucovorin, megestrol, mesna, methotrexate, metoclopramide, mitomycin, mitotane, mitoxantrone, omeprazole, ondansetron, paclitaxel (Taxol^{TM}), pilocarpine, prochloroperazine, rituximab, saproin, tamoxifen, taxol, topotecan hydrochloride, trastuzumab, vinblastine, vincristine and vinorelbine tartrate.

[0056] "Concomitant administration" of a known cancer therapeutic drug with a pharmaceutical composition of the present invention means administration of the drug and SIRPabody at such time that both the known drug and the composition of the present invention will have a therapeutic effect. Such concomitant administration may involve concurrent (*i.e.* at the same time), prior, or subsequent administration of the drug with respect to the administration of a compound of the invention. A person of ordinary skill in the art

would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present invention.

[0057] As used herein, the phrase “disease-free survival,” refers to the lack of such tumor recurrence and/or spread and the fate of a patient after diagnosis, with respect to the effects of the cancer on the life-span of the patient. The phrase “overall survival” refers to the fate of the patient after diagnosis, despite the possibility that the cause of death in a patient is not directly due to the effects of the cancer. The phrases, “likelihood of disease-free survival”, “risk of recurrence” and variants thereof, refer to the probability of tumor recurrence or spread in a patient subsequent to diagnosis of cancer, wherein the probability is determined according to the process of the invention.

[0058] As used herein, the term “correlates,” or “correlates with,” and like terms, refers to a statistical association between instances of two events, where events include numbers, data sets, and the like. For example, when the events involve numbers, a positive correlation (also referred to herein as a “direct correlation”) means that as one increases, the other increases as well. A negative correlation (also referred to herein as an “inverse correlation”) means that as one increases, the other decreases.

[0059] "Dosage unit" refers to physically discrete units suited as unitary dosages for the particular individual to be treated. Each unit can contain a predetermined quantity of active compound(s) calculated to produce the desired therapeutic effect(s) in association with the required pharmaceutical carrier. The specification for the dosage unit forms can be dictated by (a) the unique characteristics of the active compound(s) and the particular therapeutic effect(s) to be achieved, and (b) the limitations inherent in the art of compounding such active compound(s).

[0060] "Pharmaceutically acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

[0061] "Pharmaceutically acceptable salts and esters" means salts and esters that are pharmaceutically acceptable and have the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the compounds are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, *e.g.* sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, *e.g.*, ethanolamine, diethanolamine, triethanolamine, tromethamine, N methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (*e.g.*, hydrochloric and hydrobromic acids) and organic acids (*e.g.*, acetic acid, citric acid, maleic acid, and the

alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). Pharmaceutically acceptable esters include esters formed from carboxy, sulfonyloxy, and phosphonoxy groups present in the compounds, *e.g.*, C_{1-6} alkyl esters. When there are two acidic groups present, a pharmaceutically acceptable salt or ester can be a mono-acid-mono-salt or ester or a di-salt or ester; and similarly where there are more than two acidic groups present, some or all of such groups can be salified or esterified. Compounds named in this invention can be present in unsalified or unesterified form, or in salified and/or esterified form, and the naming of such compounds is intended to include both the original (unsalified and unesterified) compound and its pharmaceutically acceptable salts and esters. Also, certain compounds named in this invention may be present in more than one stereoisomeric form, and the naming of such compounds is intended to include all single stereoisomers and all mixtures (whether racemic or otherwise) of such stereoisomers.

[0062] The terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a human without the production of undesirable physiological effects to a degree that would prohibit administration of the composition.

[0063] As used herein, the term “antibody” refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. As is known in the art, intact antibodies as produced in nature are approximately 150 kD tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)– an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y’s stem). A short region, known as the “switch”, connects the heavy chain variable and constant regions. The “hinge” connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another “switch”. Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain.

[0064] Each domain in a natural antibody has a structure characterized by an "immunoglobulin fold" formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as "complement determining regions" (CDR1, CDR2, and CDR3) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity.

[0065] As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, SIRPabodies produced and/or utilized in accordance with the present invention may include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation. For purposes of the present invention, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an "antibody", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology.

[0066] In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc, as is known in the art. Moreover, the term "antibody" as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgG, IgE and IgM, bi- or multi- specific antibodies (e.g., Zybodies®, etc), single chain Fvs, polypeptide-Fc fusions, Fabs, cameloid antibodies, masked antibodies (e.g., Probodies®), Small Modular ImmunoPharmaceuticals ("SMIPs™"), single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, minibodies, BiTE®s, ankyrin repeat proteins or DARPINs®, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Fynomers®, Centyrins®, and a KALBITOR®.

[0067] As used herein, the term "antibody-dependent cellular cytotoxicity" or "ADCC" refers to a phenomenon in which target cells bound by antibody are killed by immune effector cells.

Without wishing to be bound by any particular theory, ADCC is typically understood to involve Fc receptor (FcR)-bearing effector cells can recognizing and subsequently killing antibody-coated target cells (e.g., cells that express on their surface specific antigens to which an antibody is bound). Effector cells that mediate ADCC can include immune cells, including but not limited to one or more of natural killer (NK) cells, macrophage, neutrophils, eosinophils.

[0068] The term "antigen", as used herein, refers to an agent that elicits an immune response; and/or for the purposes of the invention, (ii) an agent that binds to an antibody. In general, an antigen may be or include any chemical entity such as, for example, a small molecule, a nucleic acid, a polypeptide, a carbohydrate, a lipid, a polymer (in some embodiments other than a biologic polymer [e.g., other than a nucleic acid or amino acid polymer) etc. In some embodiments, an antigen is or comprises a polypeptide. In some embodiments, an antigen is or comprises a glycan. Those of ordinary skill in the art will appreciate that, in general, an antigen may be provided in isolated or pure form, or alternatively may be provided in crude form (e.g., together with other materials, for example in an extract such as a cellular extract or other relatively crude preparation of an antigen-containing source).

[0069] The term "*anti-CD20 antibody*" refers herein to monoclonal or polyclonal antibodies with specificity for the polypeptide CD20. Antibodies with specificity for CD20 can be prepared by methods that are well understood in the art. Preferred antibody compositions are ones that have been selected for antibodies directed against a polypeptide or polypeptides of CD20. Particularly preferred polyclonal antibody preparations are ones that contain only antibodies directed against a polypeptide or polypeptides of CD20. Anti-CD20 antibodies that are suitable for use in the current invention would include, for example, rituximab, ibritumomab tiuxetan, tositumomab, AME-133v (Applied Molecular Evolution), Ocrelizumab (Roche), Ofatumumab (Genmab), TRU-015 (Trubion) and IMMU-106 (Immunomedics). In one embodiment, the anti-CD20 antibody is rituximab.

[0070] "*CD20", "CD20 protein*", and "*CD20 polypeptide*" are used interchangeably herein to refer to a polypeptide encoded by a member of the membrane-spanning 4A gene family. This gene, referred to as "MS4A1", "Membrane-spanning 4-domains, subfamily A, member 1","B1" and "B-lymphocyte surface antigen B1", encodes a non-glycosylated phosphoprotein of 297 amino acids, as described at, for example, Genbank NM_152866 and Genbank NM_021950 (alternative splice variants that encode the same protein). CD20 polypeptide is expressed on the surface of B cells beginning at the late pre-B cell phase of development, and plays a role in B cell proliferation.

[0071] Antibodies targeting tumor antigens have been approved for use in treating cancers, and are rapidly becoming standard of care. A non-comprehensive list of certain human

antigens targeted by known, available antibody agents, and notes certain cancer indications for which the antibody agents have been proposed to be useful:

CD2	Siplizumab	Non-Hodgkin's Lymphoma
CD3	UCHT1	Peripheral or Cutaneous T-cell Lymphoma
CD4	HuMax-CD4	
CD19	SAR3419, MEDI-551	Diffuse Large B-cell Lymphoma
CD19 and CD3 or CD22	Bispecific antibodies such as Blinatumomab, DT2219ARL	Non-Hodgkin's Lymphoma
CD20	Rituximab, Veltuzumab, Tositumomab, Ofatumumab, Ibritumomab, Obinutuzumab,	B cell malignancies (Non-Hodgkin's lymphoma, Chronic lymphocytic leukemia)
CD22 (SIGLEC2)	Inotuzumab, tetraxetan,CAT-8015, DCDT2980S, Bectumomab	Chemotherapy-resistant hairy cell leukemia, Hodgkin's lymphoma
CD30	Brentuximab vedotin	
CD33	Gemtuzumab ozogamicin (Mylotarg)	Acute myeloid leukemia
CD37	TRU-016	Chronic lymphocytic leukemia
CD38	Daratumumab	Multiple myeloma, hematological tumors
CD40	Lucatumumab	Non-Hodgkin's lymphoma
CD52	Alemtuzumab (Campath)	Chronic lymphocytic leukemia
CD56 (NCAM1)	Lorvotuzumab	Small Cell Lung Cancer
CD66e (CEA)	Labetuzumab	Breast, colon and lung tumors
CD70	SGN-75	Non-Hodgkin's lymphoma
CD74	Milatuzumab	Non-Hodgkin's lymphoma
CD138 (SYND1)	BT062	Multiple Myeloma
CD152 (CTLA-4)	Ipilimumab	Metastatic melanoma
CD221 (IGF1R)	AVE1642, IMC-A12, MK-0646, R150, CP 751871	Glioma, lung, breast, head and neck, prostate and thyroid cancer
CD254 (RANKL)	Denosumab	Breast and prostate carcinoma
CD261 (TRAILR1)	Mapatumumab	Colon, lung and pancreas tumors and haematological malignancies
CD262 (TRAILR2)	HGS-ETR2, CS-1008	
CD326 (Epcam)	Edrecolomab, 17-1A, IGN101, Catumaxomab, Adecatumumab	Colon and rectal cancer, malignant ascites, epithelial tumors (breast, colon, lung)
CD309 (VEGFR2)	IM-2C6, CDP791	Epithelium-derived solid tumors
CD319 (SLAMF7)	HuLuc63	Multiple myeloma
CD340 (HER2)	Trastuzumab, Pertuzumab, Ado-trastuzumab emtansine	Breast cancer
CAIX (CA9)	cG250	Renal cell carcinoma
EGFR (c-erbB)	Cetuximab, Panitumumab, nimotuzumab and 806	Solid tumors including glioma, lung, breast, colon, and head and neck tumors
EPHA3 (HEK)	KB004, IIIA4	Lung, kidney and colon tumors, melanoma, glioma and haematological malignancies
Episialin	Epitumomab	Epithelial ovarian tumors
FAP	Sibrotuzumab and F19	Colon, breast, lung, pancreas, and head and neck tumors
HLA-DR beta	Apolizumab	Chronic lymphocytic leukemia, non-Hodkin's lymphoma

FOLR-1	Farletuzumab	Ovarian tumors
5T4	Anatumomab	Non-small cell lung cancer
GD3/GD2	3F8, ch14.18, KW-2871	Neuroectodermal and epithelial tumors
gpA33	huA33	Colorectal carcinoma
GPNMB	Glembatumumab	Breast cancer
HER3 (ERBB3)	MM-121	Breast, colon, lung, ovarian, and prostate tumors
Integrin αVβ3	Etaracizumab	Tumor vasculature
Integrin α5β1	Volociximab	Tumor vasculature
Lewis-Y antigen	hu3S193, IgN311	Breast, colon, lung and prostate tumors
MET (HGFR)	AMG 102, METMAB, SCH900105	Breast, ovary and lung tumors
Mucin-1/CanAg	Pemtumomab, oregovomab, Cantuzumab	Breast, colon, lung and ovarian tumors
PSMA	ADC, J591	Prostate Cancer
Phosphatidylserine	Bavituximab	Solid tumors
TAG-72	Minretumomab	Breast, colon and lung tumors
Tenascin	81C6	Glioma, breast and prostate tumours
VEGF	Bevacizumab	Tumour vasculature

[0072] As used herein, the term “therapeutically effective amount” means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, and/or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence and/or severity of, stabilizes one or more characteristics of, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that the term “therapeutically effective amount” does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment.

[0073] For example, in some embodiments, the term "therapeutically effective amount", refers to an amount which, when administered to an individual in need thereof in the context of inventive therapy, will block, stabilize, attenuate, or reverse a cancer-supportive process occurring in said individual, or will enhance or increase a cancer-suppressive process in said individual. In the context of cancer treatment, a "therapeutically effective amount" is an amount which, when administered to an individual diagnosed with a cancer, will prevent, stabilize, inhibit, or reduce the further development of cancer in the individual. A particularly preferred "therapeutically effective amount" of a composition described herein reverses (in a therapeutic treatment) the development of a malignancy, such as a B cell lymphoma, or helps achieve or prolong remission of a malignancy.

[0074] A therapeutically effective amount administered to an individual to treat a cancer in that individual may be the same or different from a therapeutically effective amount administered to promote remission or inhibit metastasis. As with most cancer therapies, the therapeutic methods described herein are not to be interpreted as, restricted to, or otherwise limited to a "cure" for cancer; rather the methods of treatment are directed to the use of the described compositions to "treat" a cancer, i.e., to effect a desirable or beneficial change in the health of an individual who has cancer. Such benefits are recognized by skilled healthcare providers in the field of oncology and include, but are not limited to, a stabilization of patient condition, a decrease in tumor size (tumor regression), an improvement in vital functions (e.g., improved function of cancerous tissues or organs), a decrease or inhibition of further metastasis, a decrease in opportunistic infections, an increased survivability, a decrease in pain, improved motor function, improved cognitive function, improved feeling of energy (vitality, decreased malaise), improved feeling of well-being, restoration of normal appetite, restoration of healthy weight gain, and combinations thereof.

[0075] In addition, regression of a particular tumor in an individual (e.g., as the result of treatments described herein) may also be assessed by taking samples of cancer cells from the site of a tumor such as a B cell lymphoma (e.g., over the course of treatment) and testing the cancer cells for the level of metabolic and signaling markers to monitor the status of the cancer cells to verify at the molecular level the regression of the cancer cells to a less malignant phenotype. For example, tumor regression induced by employing the methods of this invention would be indicated by finding a decrease in any of the pro-angiogenic markers, an increase in anti-angiogenic markers, the normalization (i.e., alteration toward a state found in normal individuals not suffering from cancer) of metabolic pathways, intercellular signaling pathways, or intracellular signaling pathways that exhibit abnormal activity in individuals diagnosed with cancer. Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount may be formulated and/or administered in a single dose. In some embodiments, a therapeutically effective amount may be formulated and/or administered in a plurality of doses, for example, as part of a dosing regimen.

METHODS OF USE

[0076] The present invention provides methods for treating, reducing or preventing cancer, including without limitation hematopoietic cancers, and metastasis of cancers, by inhibiting the interaction between SIRPα and CD47 in a targeted manner, thereby increasing phagocytosis of tumor cells. Such methods include administering to a subject in need of treatment a therapeutically effective amount or an effective dose of a SIRPabody, where there immunoglobulin variable region of the SIRPabody specifically binds a cell surface protein on the tumor cell, particularly binding to CD20. Effective doses of the therapeutic entity of the

present invention, *e.g.* for the treatment of cancer, vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human, but nonhuman mammals may also be treated. Treatment dosages need to be titrated to optimize safety and efficacy.

[0077] In some embodiments, the dosage may range from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once every two weeks or once a month or once every 3 to 6 months. Therapeutic entities of the present invention are usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the therapeutic entity in the patient. Alternatively, therapeutic entities of the present invention can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the polypeptide in the patient.

[0078] In prophylactic applications, a relatively low dosage may be administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In other therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

[0079] In still other embodiments, methods of the present invention include treating, reducing or preventing tumor growth, tumor metastasis or tumor invasion of cancers including lymphomas, leukemias, carcinomas, melanomas, glioblastomas, sarcomas, myelomas, *etc.* For prophylactic applications, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of disease in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease.

[0080] Compositions for the treatment of cancer can be administered by parenteral, topical, intravenous, intratumoral, oral, subcutaneous, intraarterial, intracranial, intraperitoneal, intranasal or intramuscular means. A typical route of administration is intravenous or intratumoral, although other routes can be equally effective.

[0081] Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to

injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above. Langer, Science 249: 1527, 1990 and Hanes, Advanced Drug Delivery Reviews 28: 97-119, 1997. The agents of this invention can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient. The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[0082] Toxicity of the proteins described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD_{50} (the dose lethal to 50% of the population) or the LD_{100} (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the proteins described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition.

[0083] In one aspect, the invention provides SIRPabody polypeptides, and isolated nucleic acids encoding SIRPabody polypeptides. For recombinant production of the SIRPabody, the nucleic acid encoding it is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

[0084] The SIRPabody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for human γ3 (Guss et al., EMBO J.

5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH_3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0085] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

[0086] Therapeutic formulations comprising one or more SIRPabodies of the invention are prepared for storage by mixing the SIRPabody having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. The antibody composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent the CD47 associated disease.

[0087] The therapeutic dose may be at least about 0.01 μg/kg body weight, at least about 0.05 μg/kg body weight; at least about 0.1 μg/kg body weight, at least about 0.5 μg/kg body weight, at least about 1 μg/kg body weight, at least about 2.5 μg/kg body weight, at least about 5 μg/kg body weight, and not more than about 100 μg/kg body weight. It will be understood by one of skill in the art that such guidelines will be adjusted for the molecular weight of the active agent, *e.g.* in the use of antibody fragments, or in the use of antibody conjugates. The dosage may also be varied for localized administration, *e.g.* intranasal, inhalation, *etc.*, or for systemic administration, *e.g.* i.m., i.p., i.v., and the like.

[0088] The antibody need not be, but is optionally formulated with one or more agents that potentiate activity, or that otherwise increase the therapeutic effect. These are generally used

in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[0089] Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyidimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0090] The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0091] In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the SIRPabody. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user

standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0092] Also within the scope of the invention are kits comprising the compositions (*e.g.*, SIRPabodies and formulations thereof) of the invention and instructions for use. The kit can further contain a least one additional reagent, e.g. a chemotherapeutic drug, *etc.* Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

[0093] The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

EXPERIMENTAL

[0094] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.* amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0095] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0096] The present invention has been described in terms of particular embodiments found or proposed by the present inventor to comprise preferred modes for the practice of the invention. It will be appreciated by those of skill in the art that, in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments exemplified without departing from the intended scope of the invention. For example, due to codon redundancy, changes can be made in the underlying DNA sequence without affecting the protein sequence. Moreover, due to biological functional equivalency considerations, changes can be made in protein structure without affecting the biological action in kind or amount. All such modifications are intended to be included within the scope of the appended claims.

Example 1

[0097] SIRPabodies were created with the aim of co-targeting CD47 and a second antigen. CD20 was chosen for this purpose. The N-terminal immunoglobulin fold from wild type human SIRPα was engineered onto either the N or C terminus of the heavy chain of rituximab, an established anti-CD20 antibody (Figure 1A, 1B).

[0098] Production of recombinant SIRPα-rituximab bispecific reagents was confirmed by analysis of purified protein on reducing and non-reducing SDS-PAGE (Figure 2).

[0099] Each SIRPabody (engineered bispecific variant) retained the ability to bind the CD20 antigen expressed on the cell surface (Figure 3). Accordingly, when CD20-expressing cells were stained with SIRPabodies prior to incubation with DyLight 488anti-CD20, all antibodies masked the CD20 epitope preventing subsequent binding with DyLight 488anti-CD20 (Figure 4).

[00100] All SIRPabody reagents were able to bind to CD47, although the strength of binding varied between formats (Figure 5).

[00101] When CD47-expressing cells were first stained with SIRPabodies, subsequent incubation with labeled anti-CD47 outcompeted the primary SIRPabody staining, indicating a weak affinity of each SIRPabodies for CD47 (Figure 6). Weak binding to CD47 is a desired characteristic for SIRPabodies, as those reagents will require the avidity contributions from interactions with CD20 for binding to dual antigen cells.

[00102] To further explore the affinity of SIRPabodies to CD47, ELISA assays were performed to measure binding to recombinant CD47 antigen (Figure 7). Consistently, all variants exhibited reduced affinity for CD47 relative to monoclonal anti-CD47. The affinity of each SIRPabody for CD47 was calculated using surface plasmon resonance measurements and relative affinities were consistent with all previous assays, indicating a reduced affinity for CD47 compared to monoclonal anti-CD47 (Figure 8).

[00103] To assess whether SIRPabodies are capable of simultaneous binding to CD20 and CD47, antibodies were coincubated with CD20+CD47- cells and fluorescent NeutrAvidin coated with biotinylated CD47 fusion protein. SIRPabody binding was detected with secondary antibody and double positive fluorescent events indicated simultaneous binding to each antigen by the primary antibody (Figure 9). Binding to both CD20 and CD47 on the cell surface was demonstrated by incubating cells expressing both antigens with SIRPabodies prior to staining with APC anti-CD47 or DyLight 488anti-CD20. All bispecific reagents blocked the subsequent binding of labeled antibody indicating binding to both antigens by the primary antibody stain (Figure 10).

[00104] To determine whether the SIRPabodies have the desired selectivity for dual antigen cells in the presence of challenge with excess CD47-only expressing cells, CD20+CD47+ Raji cells were mixed with 20-fold excess red blood cells (RBCs) (CD47+CD20-). Cell mixtures

were stained with SIRPabodies and antibody binding was detected with PE anti-human secondary (Figure 11). All SIRPabodies bound to dual antigen tumor cells, while binding to single antigen RBCs was absent. Moreover, binding to dual antigen tumor cells was in part mediated by binding to CD47 as indicated by the ability of primary antibody stain to block subsequent staining with APC anti-CD47 (Figure 11d).

[00105] The therapeutic mechanism of action for SIRPabodies is induction of phagocytosis. Phagocytosis was measured in vitro by coincubating human macrophages with Raji-GFP cells in the presence of antibody. Engulfment of Raji cells by macrophages was detected by flow cytometry. All SIRPabodies were capable of inducing phagocytosis (Figure 12).

[00106] CD20-2GL-SIRPα was selected as a lead candidate for further studies of therapeutic efficacy in vivo. A human lymphoma cell line engineered to express luciferase was engrafted subcutaneously into NSG mice to establish a model of localized lymphoma. Treatment with SIRPabody CD20-2GL-SIRPα resulted in elimination of the lymphoma and increased survival comparable to the synergistic effect seen with co-targeting CD20 and CD47 with combination antibody therapy.

[00107] Similar constructs were made for TIm3 and CD99. The sequence of an exemplary anti-CD99 light chain is provided in SEQ ID NO:12. The sequence of an exemplary anti-CD99 SIRPabody heavy chain is provided in SEQ ID NO:14, where the signal sequence is amino acid residues 1-19, the linker sequence is residues 471-480; and the SIRPa binding domain is residues 481-599.

What is claimed is:

1. An isolated polypeptide comprising:

an immunoglobulin variable region fused to a sequence comprising a binding domain of SIRPα.

2. The polypeptide of Claim 1, comprising:

a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of an immunoglobulin (V_L) specific for a first epitope; and

a second polypeptide comprising (i) a first domain comprising a binding region of a heavy chain variable region domain of an immunoglobulin (V_H) specific for a first epitope; and (ii) a second domain comprising the N-terminal Ig-like domain of SIRPα.

3. The polypeptide of Claim 1, comprising:

a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of an immunoglobulin (V_L) specific for a first epitope; and (ii) a second domain comprising the N-terminal Ig-like domain of SIRPα; and

a second polypeptide comprising (i) a first domain comprising a binding region of a heavy chain variable region domain of an immunoglobulin (V_H) specific for a first epitope.

4. The polypeptide of any one of Claims 1-3, wherein the first polypeptide and the second polypeptide further comprise the respective heavy and light chain constant region domains of the immunoglobulin.

5. The polypeptide of any one of Claims 1-3, wherein the first or the second polypeptide comprises the N-terminal Ig-like domain of SIRPα fused to the amino terminus of the V_L or V_H domain, respectively.

6. The polypeptide of any one of Claims 1-3, wherein the first or the second polypeptide comprises the N-terminal Ig-like domain of SIRPα fused to the carboxy terminus of the C_L or C_H domains, respectively.

7. The polypeptide of Claim 1, wherein the SIRPα domain and the V_H or C_H domains are separated by a polypeptide linker of from 1-20 amino acids in length.

8. The polypeptide of any one of Claims 1-5, comprising the amino acid sequence of SEQ ID NO:1 or a variant thereof.

9. The polypeptide of any of Claims 1-6, wherein the variable domain of the immunoglobulin specifically binds a tumor antigen.

10. The polypeptide of Claim 9, wherein the tumor antigen is CD20.

11. The polypeptide of Claim 9, wherein the tumor antigen is TIM3.

12. The polypeptide of Claim 9, wherein the tumor antigen is CD99.

13. A nucleic acid encoding a polypeptide of any one of Claims 1-12.

14. A pharmaceutical composition comprising the polypeptide of any one of Claims 1-12.

15. A method of treating cancer, the method comprising administering an effective dose of a pharmaceutical composition of Claim 14 to an individual in need thereof.

16. The method of Claim 15, wherein the cancer is a lymphoma or leukemia.

17. The method of Claim 16, wherein the cancer is a Non-Hodgkin's B cell lymphoma.

18. The method of Claim 16, wherein the cancer is chronic lymphocytic leukemia.

19. The method of Claim 16, wherein the cancer is acute myeloid leukemia.

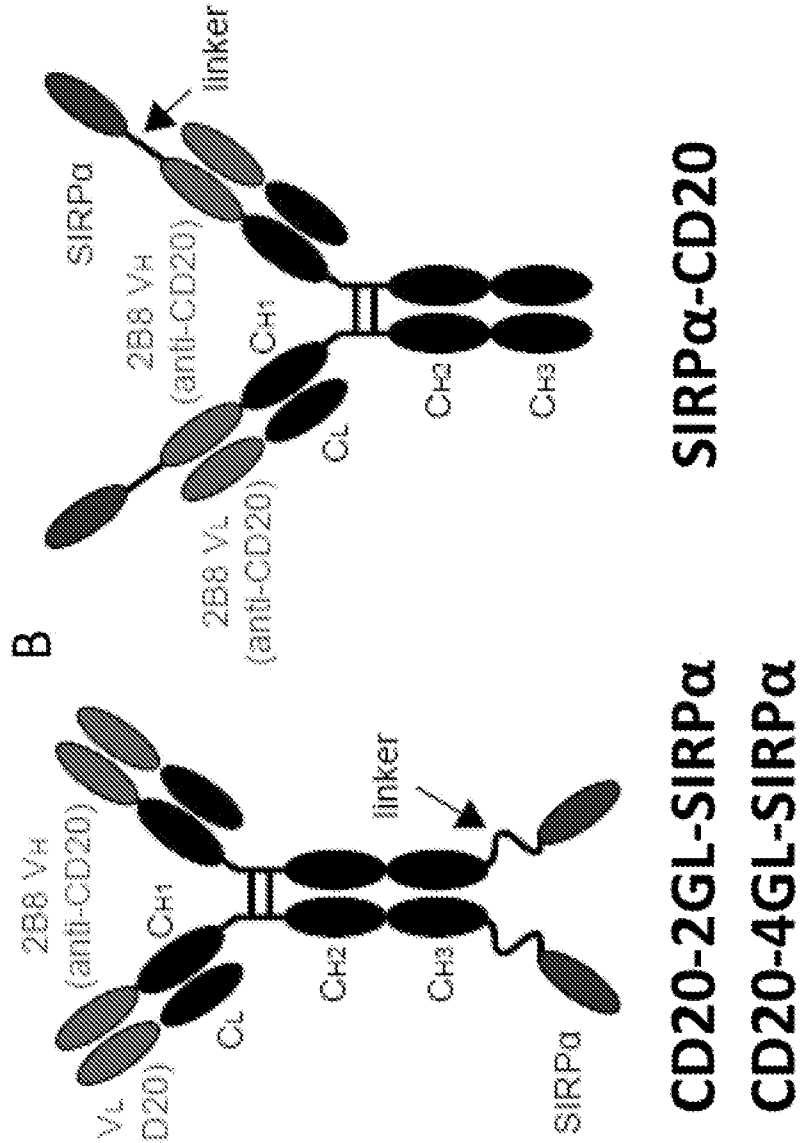
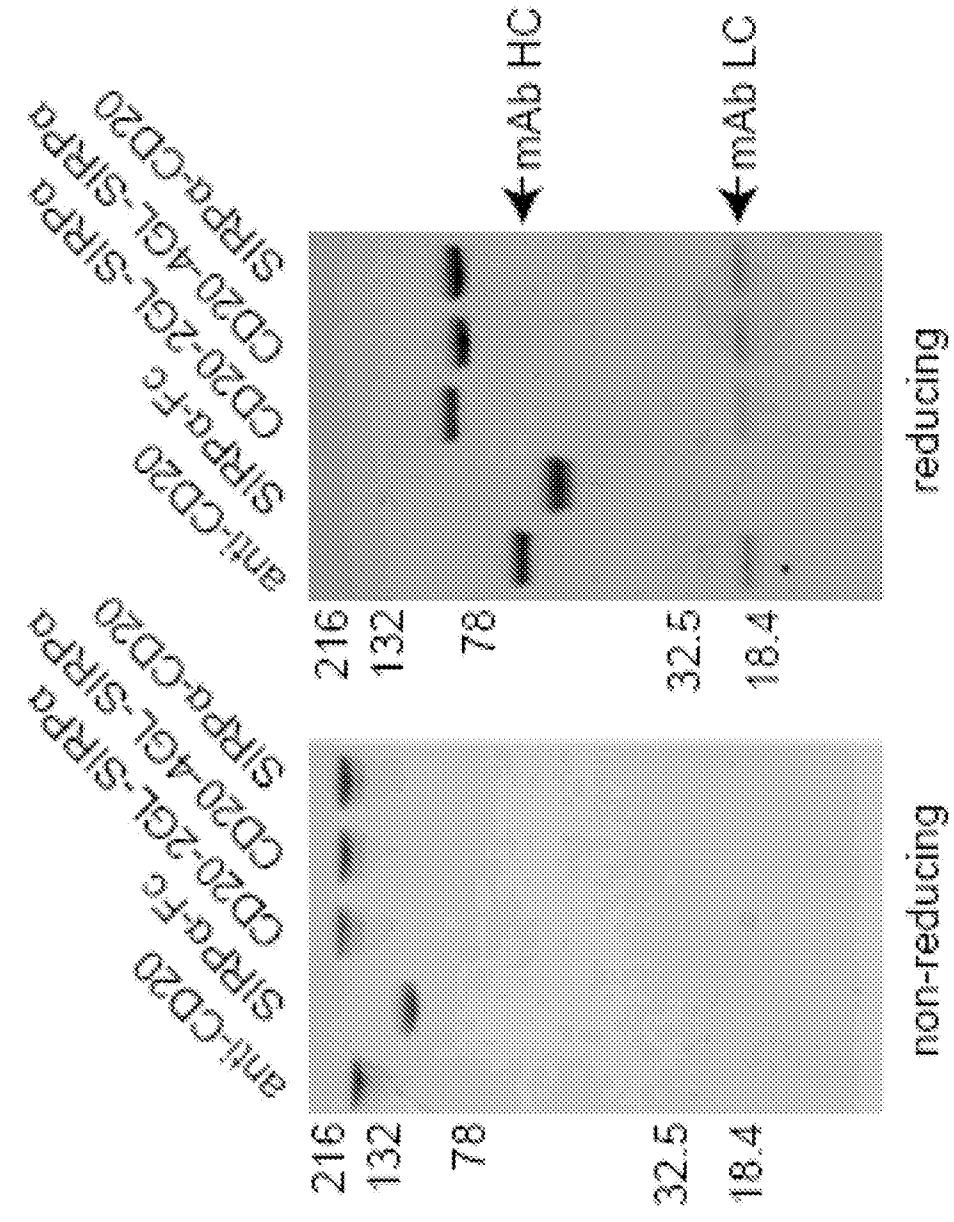

A
2B8 VH (anti-CD20)
2B8 VL (anti-CD20)
CH1
CL
CH2
CH3
linker
SIRPα
CD20-2GL-SIRPα
CD20-4GL-SIRPα
B
SIRPα
linker
2B8 VH (anti-CD20)
2B8 VL (anti-CD20)
CH1
CL
CH2
CH3
SIRPα-CD20

FIGURE 1

FIGURE 2


anti-CD20
SIRPα-Fc
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20
216
132
78
32.5
18.4
non-reducing
anti-CD20
SIRPα-Fc
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20
mAb HC
mAb LC
216
132
78
32.5
18.4
reducing

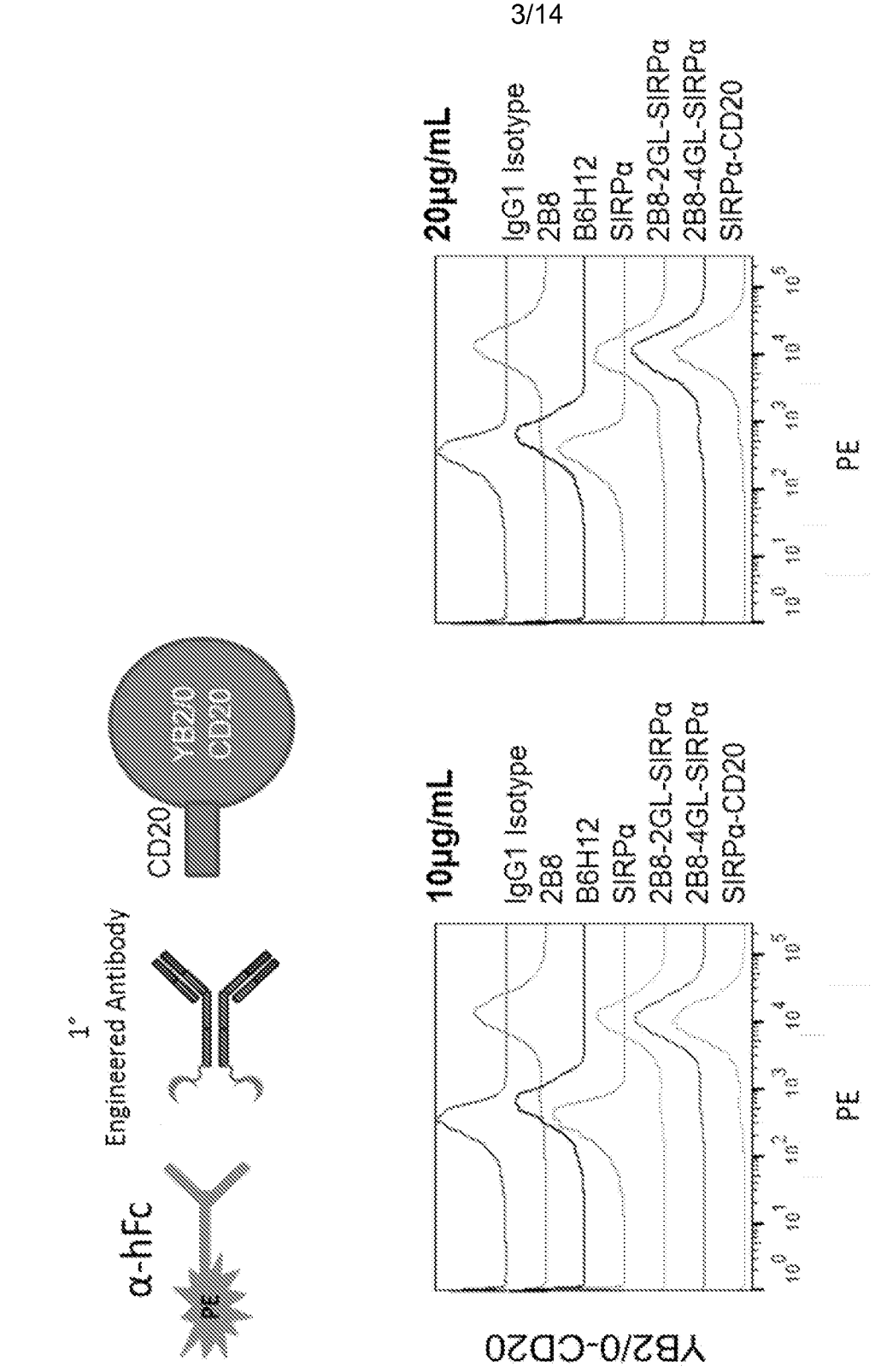

a
1°
Engineered Antibody
α-hFc
PE
CD20
YB2/0
CD20
b
YB2/0-CD20
10μg/mL
20μg/mL
IgG1 Isotype
2B8
B6H12
SIRPα
2B8-2GL-SIRPα
2B8-4GL-SIRPα
SIRPα-CD20
PE

FIGURE 3

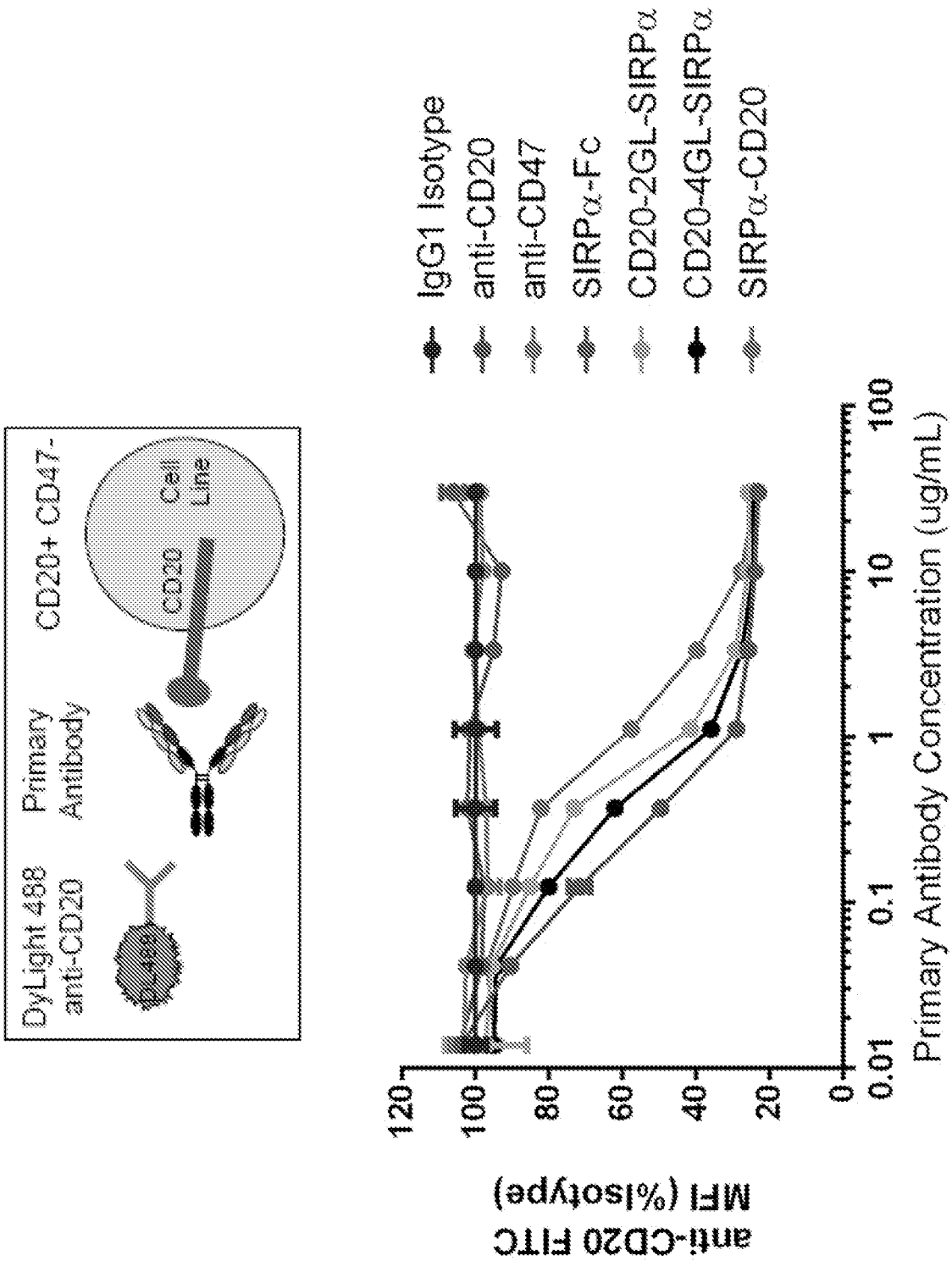

DyLight 488 anti-CD20
Primary Antibody
CD20+ CD47-
Cell Line
CD20
IgG1 Isotype
anti-CD20
anti-CD47
SIRPα-Fc
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20
anti-CD20 FITC MFI (%Isotype)
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Primary Antibody Concentration (ug/mL)

FIGURE 4

FIGURE 5

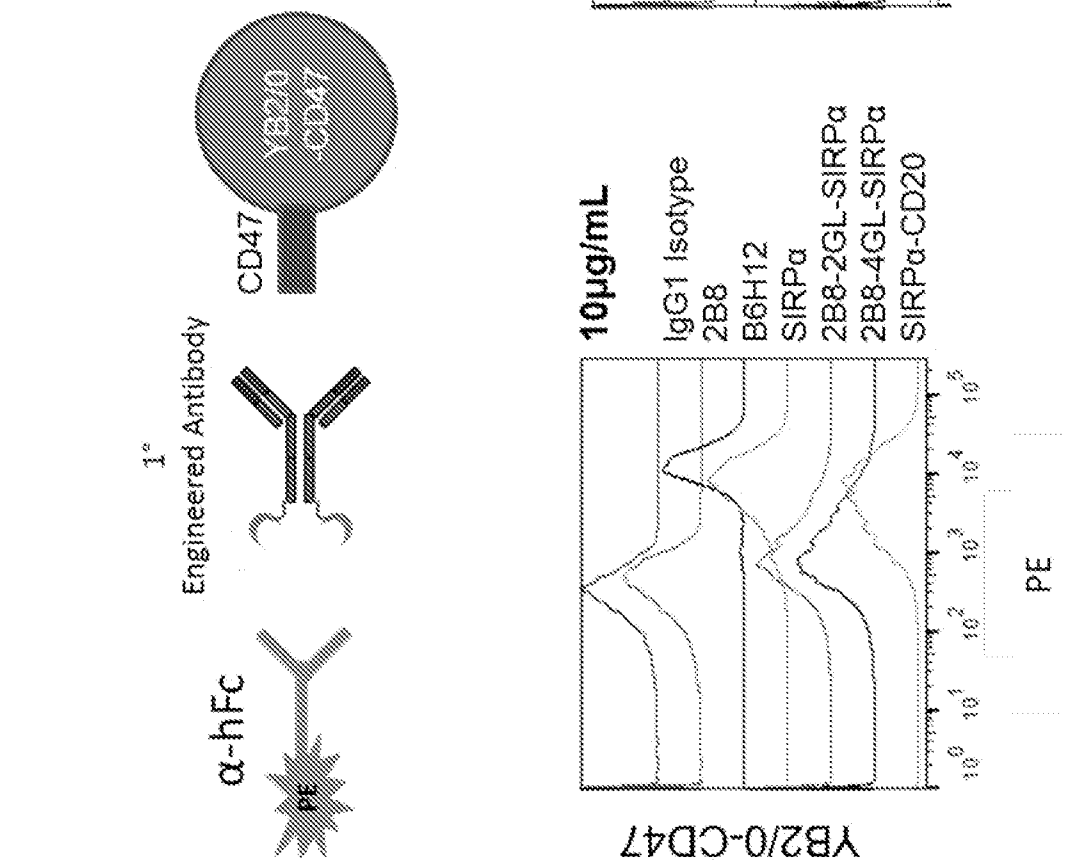
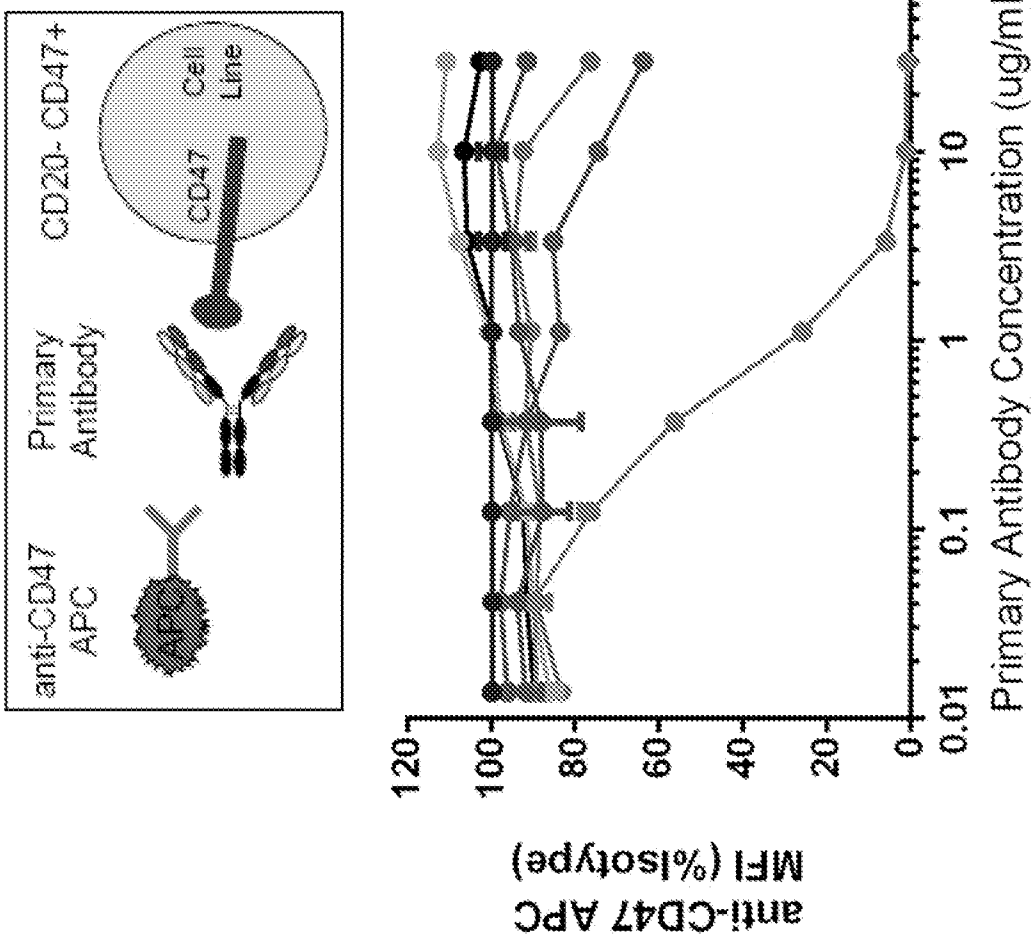

a
1°
Engineered Antibody
α-hFc
PE
CD47
YB2/0
-CD47
b
YB2/0-CD47
10μg/mL
20μg/mL
IgG1 Isotype
2B8
B6H12
SIRPα
2B8-2GL-SIRPα
2B8-4GL-SIRPα
SIRPα-CD20
PE

FIGURE 6


anti-CD47
APC
Primary
Antibody
CD20- CD47+
Cell
Line
CD47
anti-CD47 APC
MFI (%Isotype)
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Primary Antibody Concentration (ug/mL)
IgG1 Isotype
anti-CD20
anti-CD47
SIRPα-Fc
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20

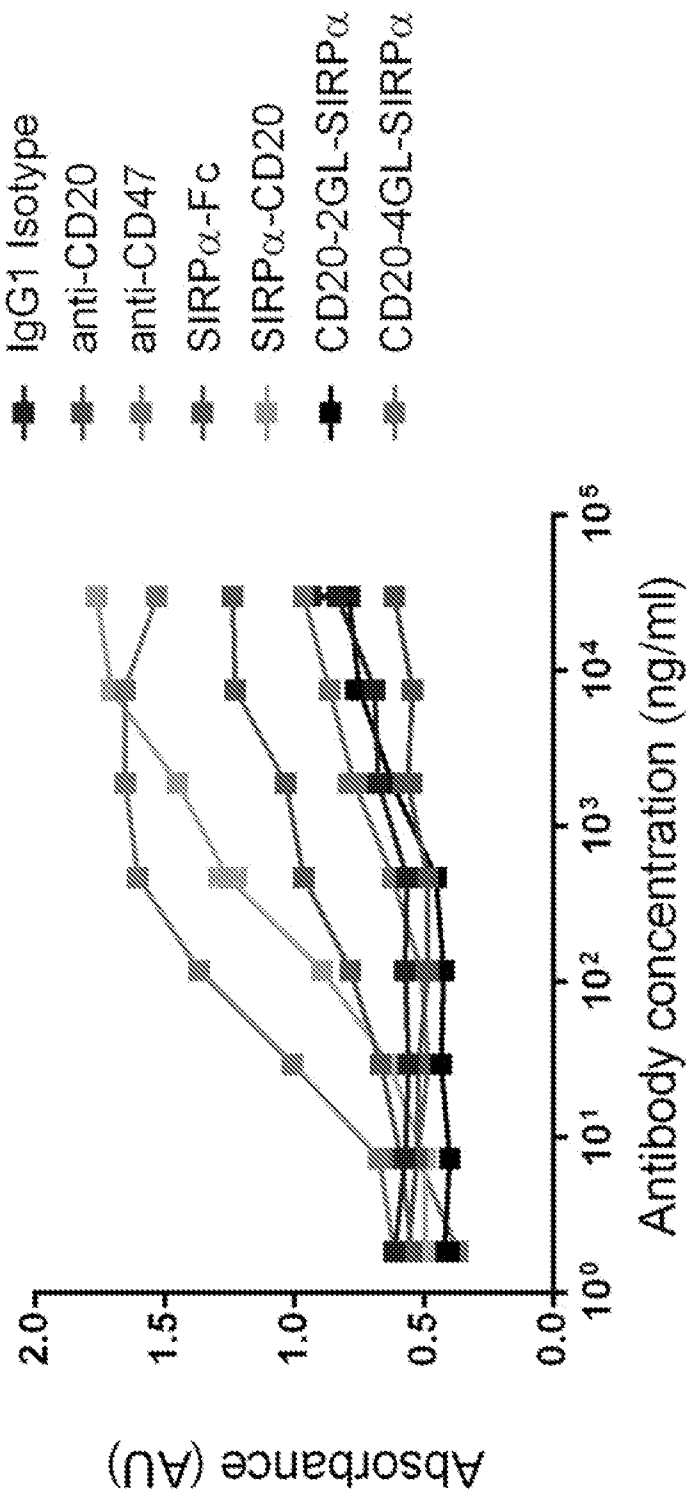
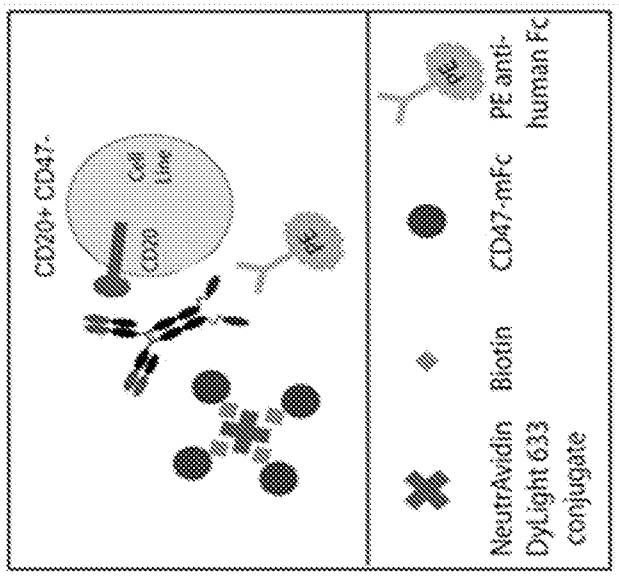

IgG1 Isotype
anti-CD20
anti-CD47
SIRPα-Fc
SIRPα-CD20
CD20-2GL-SIRPα
CD20-4GL-SIRPα
Absorbance (AU)
2.0
1.5
1.0
0.5
0.0
10⁰
10¹
10²
10³
10⁴
10⁵
Antibody concentration (ng/ml)

FIGURE 7

Antibody	K_D (nM)
Anti-CD47	0.31
SIRPα	25.4
CD20-2GL-SIRPα	21.4
CD20-4GL-SIRPα	15.93
SIRPα-CD20	6.39

FIGURE 8

FIGURE 9


a
CD20+ CD47-
Cell Line
CD20
NeutrAvidin DyLight 633 conjugate
Biotin
CD47-mFc
PE anti-human Fc

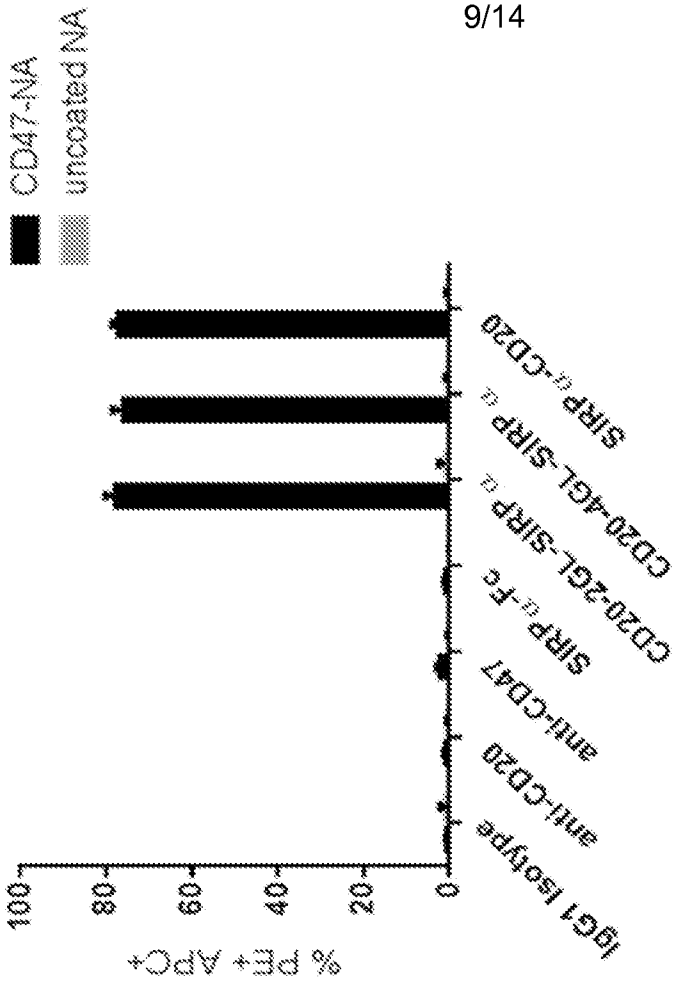

CD47-NA
uncoated NA
% PE+ APC+
0
20
40
60
80
100
IgG1 Isotype
anti-CD20
anti-CD47
SIRPα-Fc
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20

FIGURE 10

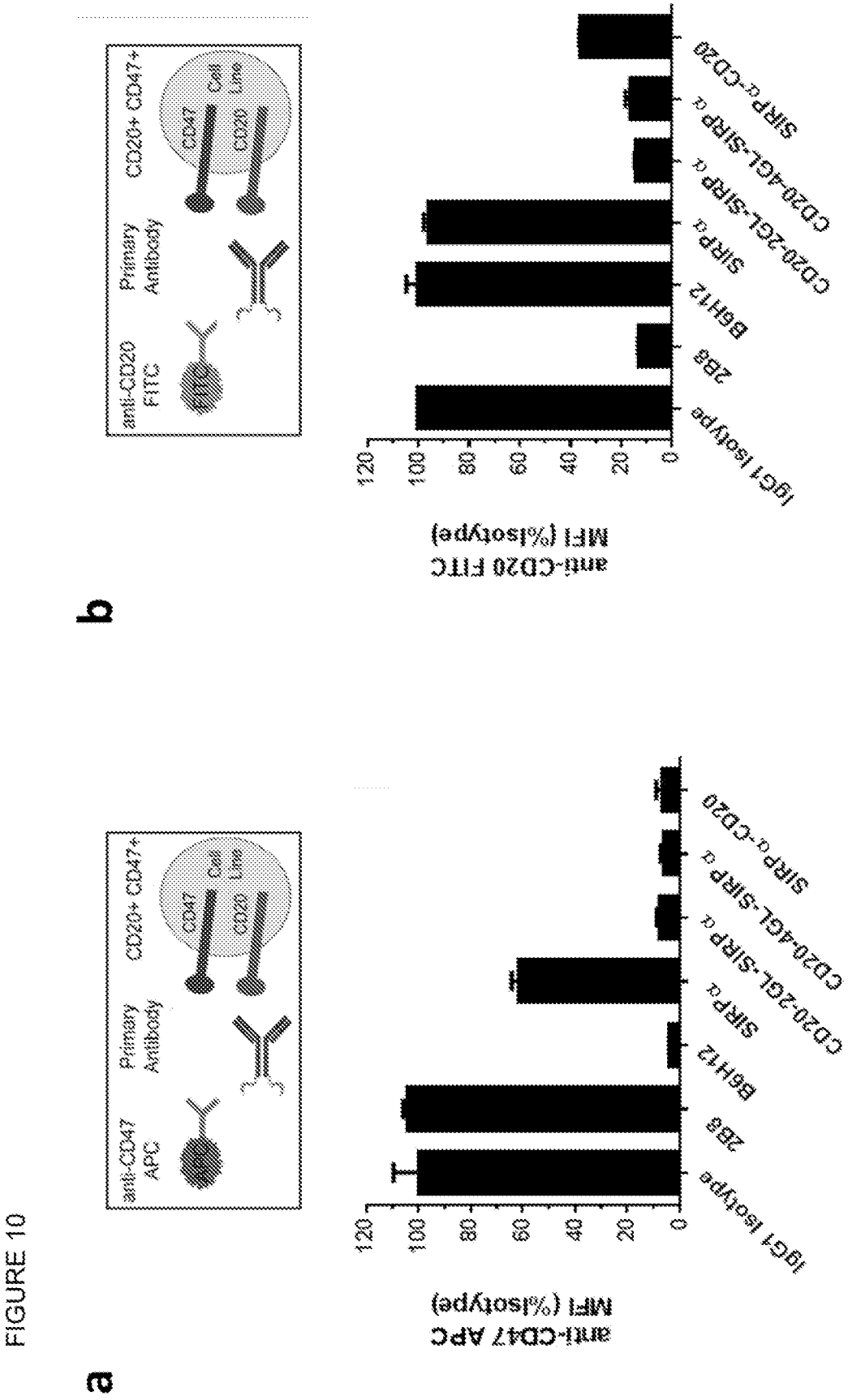
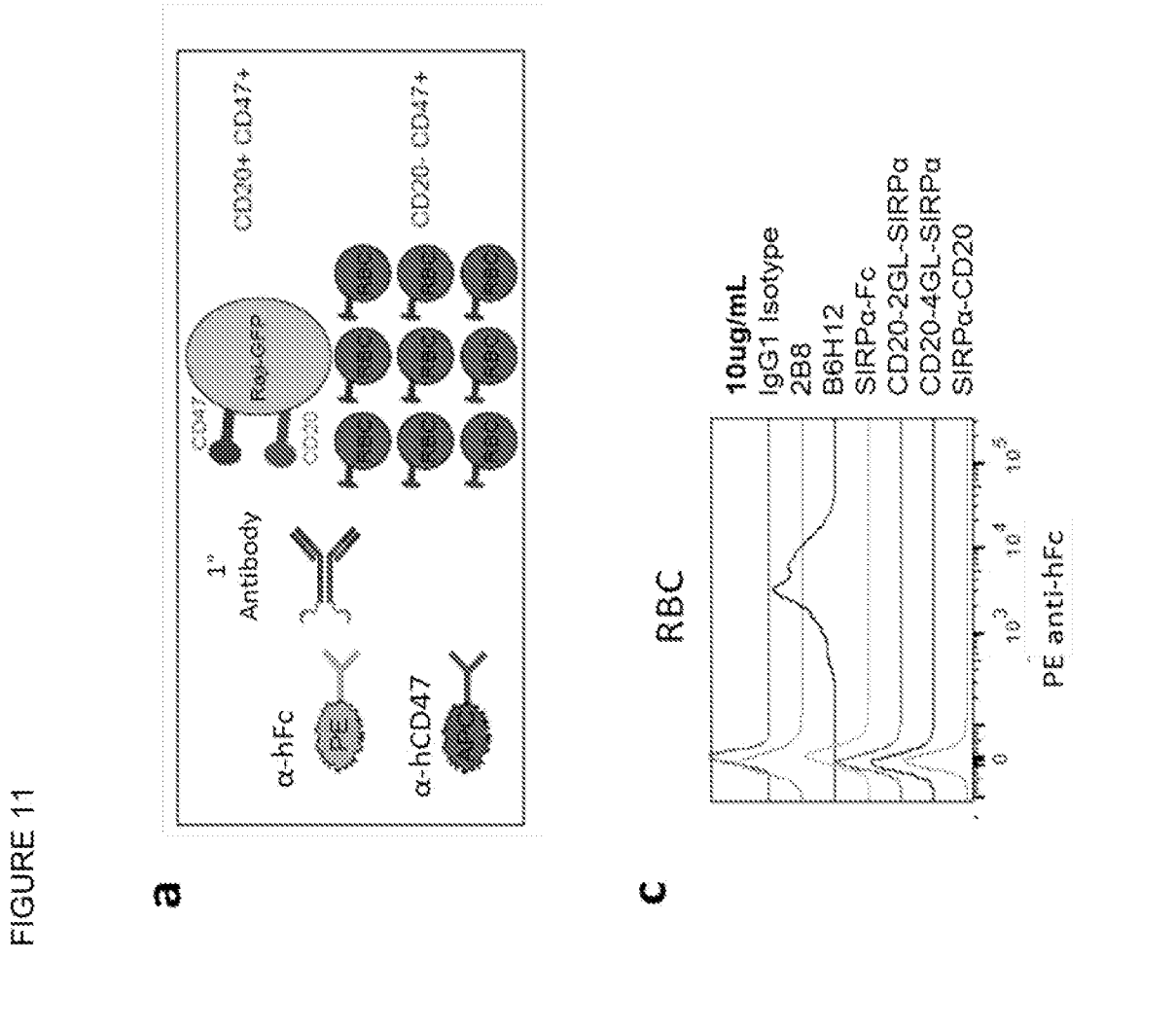

a
anti-CD47 APC
Primary Antibody
CD20+ CD47+ Cell Line
CD47
CD20
anti-CD47 APC MFI (%Isotype)
120
100
80
60
40
20
0
IgG1 Isotype
2B8
BeH12
SIRPα
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20
b
anti-CD20 FITC
Primary Antibody
CD20+ CD47+ Cell Line
CD47
CD20
anti-CD20 FITC MFI (%Isotype)
120
100
80
60
40
20
0
IgG1 Isotype
2B8
BeH12
SIRPα
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20

FIGURE 11


a
CD20+ CD47+
CD20- CD47+
1° Antibody
α-hFc
α-hCD47
b
Raji-GFP
10ug/mL
IgG1 Isotype
2B8
B6H12
SIRPα-Fc
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20
PE anti-hFc
c
RBC
10ug/mL
IgG1 Isotype
2B8
B6H12
SIRPα-Fc
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20
PE anti-hFc
d
Binding to Raji-GFP cells
anti-CD47 APC
MFI (%Isotype)
IgG1 Isotype
anti-CD20
anti-CD20
SIRP α-Fc
CD20-2GL-SIRP α
CD20-4GL-SIRP α
SIRP α-CD20

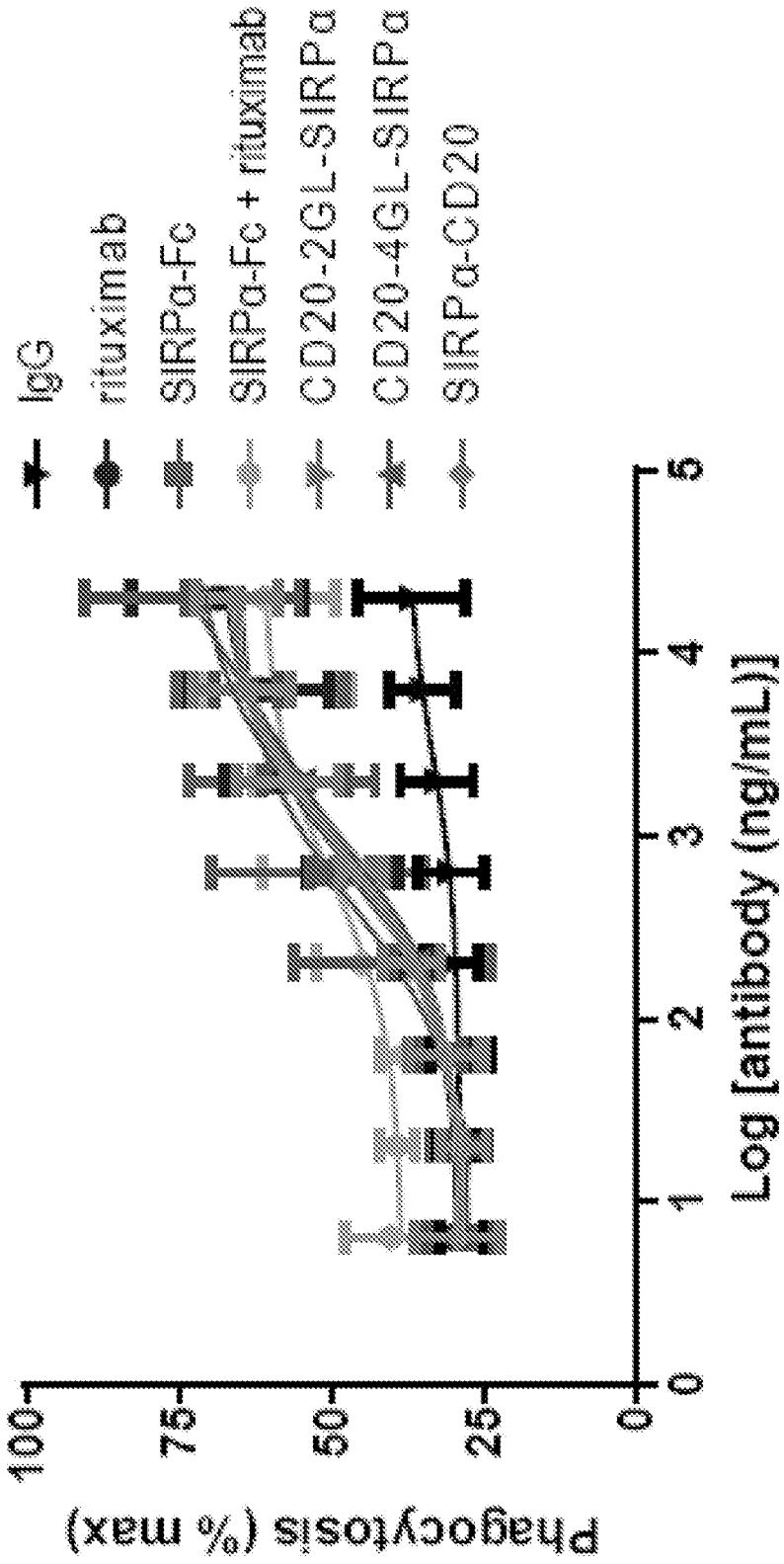

IgG
rituximab
SIRPα-Fc
SIRPα-Fc + rituximab
CD20-2GL-SIRPα
CD20-4GL-SIRPα
SIRPα-CD20
Phagocytosis (% max)
100
75
50
25
0
0
1
2
3
4
5
Log [antibody (ng/mL)]

FIGURE 12

FIGURE 13

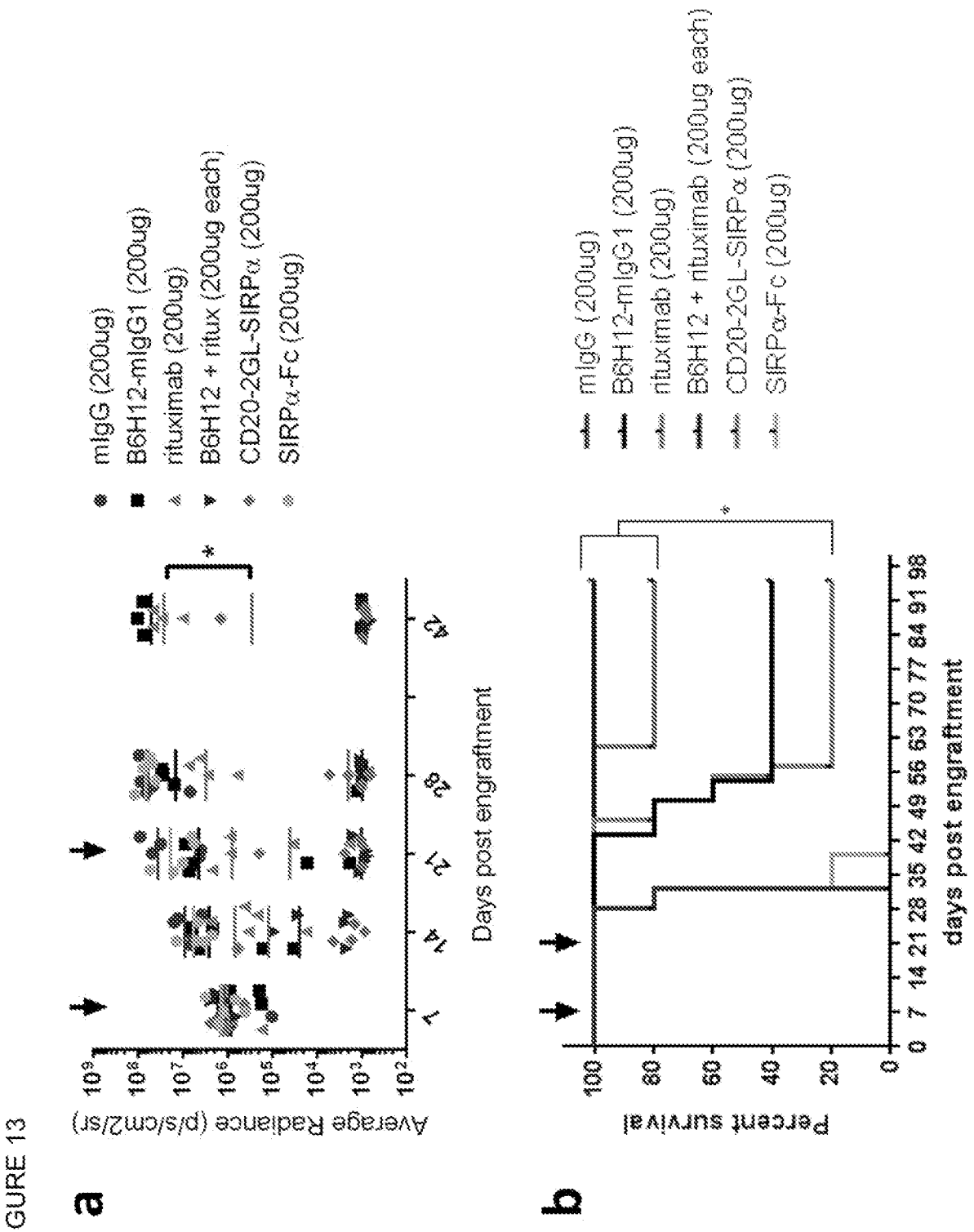
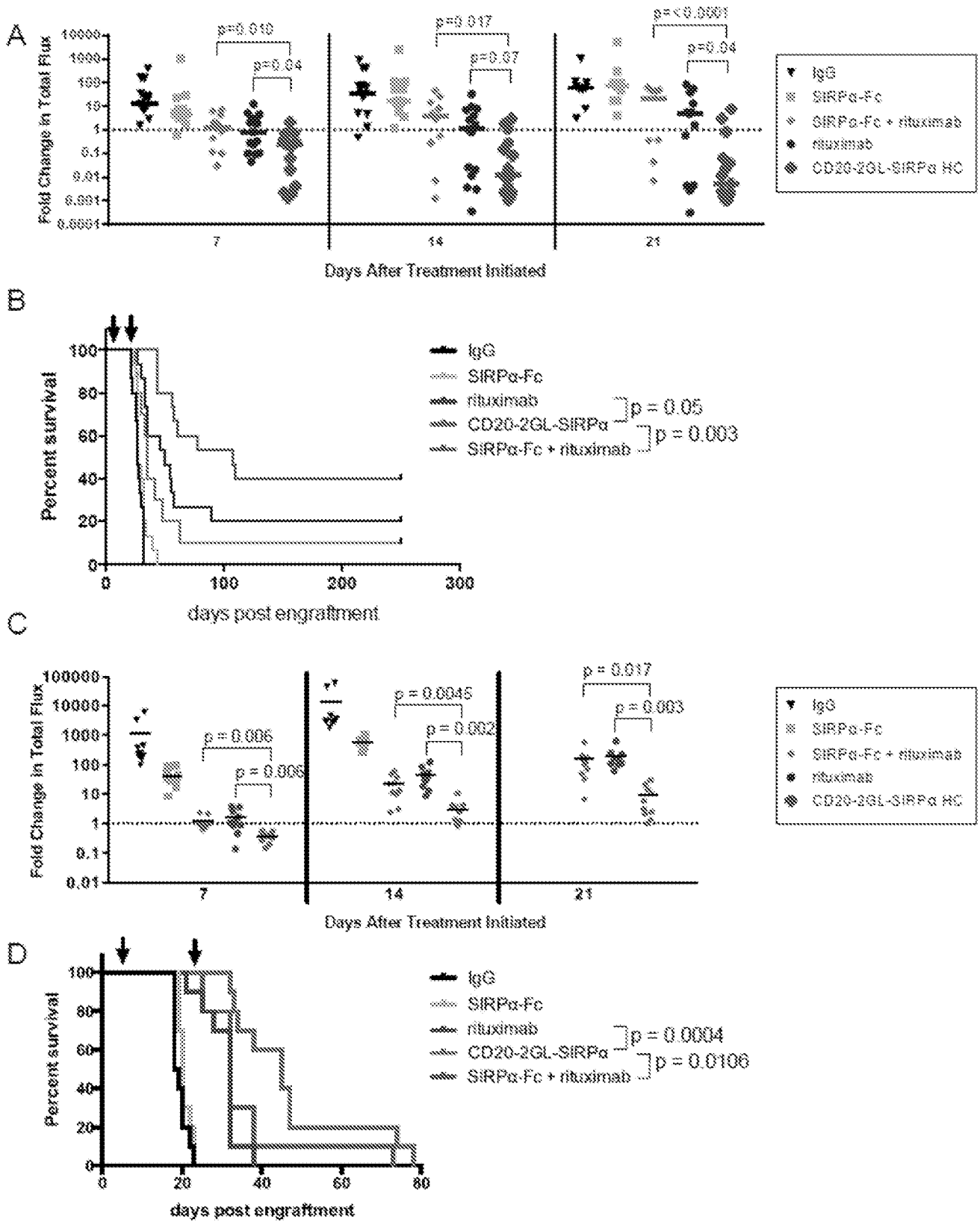

a
mIgG (200ug)
B6H12-mIgG1 (200ug)
rituximab (200ug)
B6H12 + rítux (200ug each)
CD20-2GL-SIRPα (200ug)
SIRPα-Fc (200ug)
Average Radiance (p/s/cm2/sr)
Days post engraftment
b
mIgG (200ug)
B6H12-mIgG1 (200ug)
rituximab (200ug)
B6H12 + rituximab (200ug each)
CD20-2GL-SIRPα (200ug)
SIRPα-Fc (200ug)
Percent survival
days post engraftment

FIGURE 14


A
Fold Change in Total Flux
p=0.010
p=0.04
p=0.017
p=0.07
p=<0.0001
p=0.04
IgG
SIRPα-Fc
SIRPα-Fc + rituximab
rituximab
CD20-2GL-SIRPα HC
Days After Treatment Initiated
B
Percent survival
IgG
SIRPα-Fc
rituximab
CD20-2GL-SIRPα
SIRPα-Fc + rituximab
p = 0.05
p = 0.003
days post engraftment
C
Fold Change in Total Flux
p = 0.006
p = 0.006
p = 0.0045
p = 0.002
p = 0.017
p = 0.003
IgG
SIRPα-Fc
SIRPα-Fc + rituximab
rituximab
CD20-2GL-SIRPα HC
Days After Treatment Initiated
D
Percent survival
IgG
SIRPα-Fc
rituximab
CD20-2GL-SIRPα
SIRPα-Fc + rituximab
p = 0.0004
p = 0.0106
days post engraftment

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/44304

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2015.01)
CPC - A61K 39/39558; C07K 16/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (8): A61K 38/00, 39/395; A61P 35/00; C07K 16/00 (2015.01); USPC: 514/19.3, 1.1, 1, 19.2; 424/155.1, 130.1, 152.1, 141.1, 172.1
CPC: A61K 38/00, 39/39558, 2039/505; C07K 7/06, 14/47, 16/30, 16/32, 16/18; G01N 33/57492

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; PubMed; Dialog ProQuest; immunoglobulin, antibody, 'variable region,' fused, 'SIRP-alpha,' 'signal regulatory protein alpha,' bispecific

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0193408 A1 (HUBER, T et al.) July 10, 2014; abstract; figure 1; paragraphs [0006], [0007], [0012], [0018], [0052]-[0058], [0070], [0072], [0080]-[0085], [0104], [0135], [0144]	1-5, 7 ----- 6/1-6/3
Y	US 8562997 B2 (JAISWAL, S et al.) October 22, 2013; column 12, lines 26-30; column 13, lines 59-67 to column 14, line 1	6/1-6/3
A	GUO, N et al. Engineering Bispecific Macrophage-Enhancing Antibodies As New Cancer Immunotherapies [online]. 2014 Scientific Meeting of Medical Fellows. May 2014 [retrieved on 2015-10-12]. Retrieved from the Internet: <URL: https://www.hhmi.org/sites/default/files/Programs/MedFellows/2014/2014-scientific-meeting-of-medical-fellows-meeting-book.pdf>; pages 1-61; page 18.	1-7
P, Y	PICCIONE, EC et al. A Bispecific Antibody Targeting CD47 And CD20 Selectively Binds And Eliminates Dual Antigen Expressing Lymphoma Cells. mAbs. 17 June 2015, Vol. 7, No. 5; pages 946-956; abstract. DOI: 10.1080/19420862.2015.1062192.	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2015 (13.10.2015)	09 NOV 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/44304

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-19
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.