

ANTI-SIRP α MONOCLONAL ANTIBODIES AND USES THEREOF

BACKGROUND

[0001] Signal regulatory protein alpha (SIRP α) is a member of the signal-regulatory-protein (SIRP) family, and also belongs to the immunoglobulin superfamily. SIRP family members are receptor-type transmembrane glycoproteins known to be involved in the negative regulation of receptor tyrosine kinase-coupled signaling processes. SIRP α can be phosphorylated by tyrosine kinases. The phospho-tyrosine residues of this PTP have been shown to recruit SH2 domain containing tyrosine phosphatases (PTP), and serve as substrates of PTPs. SIRP α was found to participate in signal transduction mediated by various growth factor receptors. CD47 has been demonstrated to be a ligand. SIRP α shares very high similarity with several other members of the SIRP family. SIRP α is expressed mainly by myeloid cells and also by stem cells or neurons.

[0002] SIRP α acts as inhibitory receptor and interacts with a broadly expressed transmembrane protein CD47 also called the “don’t eat me” signal. This interaction negatively controls effector function of innate immune cells such as host cell phagocytosis. SIRP α diffuses laterally on the macrophage membrane and accumulates at a phagocytic synapse to bind CD47 and signal ‘self’, which inhibits the cytoskeleton-intensive process of phagocytosis by the macrophage.

[0003] Upon CD47 ligation, SIRP α is phosphorylated and recruits phosphatases like SHP1 and SHP2. The extracellular region contains three Immunoglobulin superfamily domains - single V-set and two C1-set IgSF domains. SIRP β and γ have the similar extracellular structure but different cytoplasmic regions giving contrasting types of signals.

[0004] SIRP α recognizes CD47, an anti-phagocytic signal that distinguishes live cells from dying cells. The extracellular domain of SIRP α binds to CD47 and transmits intracellular signals through its cytoplasmic domain. CD47-binding is mediated through the NH2-terminal V-like domain of SIRP α . The cytoplasmic region contains four ITIMs that become phosphorylated after binding of ligand. The phosphorylation mediates activation of tyrosine kinase SHP2. SIRP α also binds phosphatase SHP1, adaptor protein SCAP2 and FYN-binding protein. Recruitment of SHP phosphatases to the membrane leads to the inhibition of myosin accumulation at the cell surface and results in the inhibition of phagocytosis.

[0005] Cancer cells highly express CD47 that activates SIRP α and inhibits macrophage-mediated destruction. It has been shown that high-affinity variants of SIRP α that antagonized CD47 on cancer cells increased phagocytosis of cancer cells. Anti-SIRP α antibodies have also been shown to help macrophages to reduce cancer growth and metastasis, alone and in synergy with other cancer treatments.

SUMMARY

[0006] Anti-SIRP α antibodies are discovered herein that have high affinity to both variants v1 and v2, can dose-dependently and efficiently block the interaction between SIRP α and CD47, and effectively induce macrophage mediated phagocytosis of cells expressing CD47. By contrast, known anti-SIRP α antibodies can only recognize variant 1.

[0007] In accordance with one embodiment of the present disclosure, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein (a) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20; (b) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35; (c) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52; (d) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 63, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 64, the CDRH3 comprises the amino acid sequence of SEQ ID NO:

65, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 66, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 67, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 68; (e) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 77, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 78, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 79, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 80, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 81, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 82; (f) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 91, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 92, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 93, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 94, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 95, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 96; or (g) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 103, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 104, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 105, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 106, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 107, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 108.

[0008] In one embodiment, the present disclosure provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

[0009] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27.

[0010] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29.

[0011] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:27 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:29.

[0012] In another embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0013] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44.

[0014] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46.

[0015] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:43 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:45.

[0016] Yet another embodiment provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the

antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52;

[0017] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60.

[0018] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6 and 61-62, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and 61-62.

[0019] In some embodiments, the presently disclosed antibody or fragment can bind to SIRP α variant 1 and variant 2. In some embodiments, the antibody or fragment is humanized. In some embodiments, the presently disclosed antibody or fragment further has a binding specificity to a second target protein.

[0020] Also provided, in some embodiments, are compositions comprising the antibody or fragment thereof and a pharmaceutically acceptable carrier. In some embodiments, the composition further comprises a second antibody having specificity to a tumor antigen. In some embodiments, the second antibody is a tumor-opsonizing antibody.

[0021] Methods and uses for the treatment of diseases and conditions are also provided. In one embodiment, provided is a method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 shows cross-binding to SIRP α v1 and v2 by the antibodies.

[0023] FIG. 2 shows binding affinity of the antibody against SIRP α v1.

[0024] FIG. 3 shows competition of SIRP α interaction with CD47 by the antibodies.

[0025] FIG. 4 shows induction of macrophage mediated phagocytosis by the antibodies.

[0026] FIG. 5 shows increase of macrophage mediated phagocytosis of tumor cells by the antibody treatments.

[0027] FIG. 6 presents animal scanning images and charts showing that 03-hz51 synergized with rituximab in the complete inhibition of tumor growth in Raji lymphoma tumor model.

DETAILED DESCRIPTION

Definitions

[0028] It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “an antibody,” is understood to represent one or more antibodies. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0029] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of “polypeptide,” and the term “polypeptide” may be used instead of, or interchangeably with any of these terms. The term “polypeptide” is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non- naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily

translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

[0030] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present disclosure.

[0031] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, 98 % or 99 %) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences.

[0032] The term “an equivalent nucleic acid or polynucleotide” refers to a nucleic acid having a nucleotide sequence having a certain degree of homology, or sequence identity, with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof. Likewise, “an equivalent polypeptide” refers to a polypeptide having a certain degree of homology, or sequence identity, with the amino acid sequence of a reference polypeptide. In some aspects, the sequence identity is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%. In some aspects, the equivalent polypeptide or polynucleotide has one, two, three, four or five addition, deletion, substitution and their combinations thereof as compared to the reference polypeptide or polynucleotide. In some aspects, the equivalent sequence retains the activity (*e.g.*, epitope-binding) or structure (*e.g.*, salt-bridge) of the reference sequence.

[0033] As used herein, an “antibody” or “antigen-binding polypeptide” refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof.

Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0034] The terms “antibody fragment” or “antigen-binding fragment”, as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab' , Fab , Fv , $scFv$ and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term “antibody fragment” includes aptamers, spiegelmers, and diabodies. The term “antibody fragment” also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

[0035] A “single-chain variable fragment” or “ $scFv$ ” refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L , or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. $ScFv$ molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0036] The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ , μ , α , δ , ϵ) with some subclasses among them (*e.g.*, $\gamma 1$ - $\gamma 4$). It is the nature of this chain that determines the “class” of the antibody as IgG, IgM, IgA, IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgG₅, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000

Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a “Y” configuration wherein the light chains bracket the heavy chains starting at the mouth of the “Y” and continuing through the variable region.

[0037] Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0038] Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the “tail” portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0039] Both the light and heavy chains are divided into regions of structural and functional homology. The terms “constant” and “variable” are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino- terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0040] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VK domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VK chains (*i.e.* CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. *See, e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993).

[0041] In naturally occurring antibodies, the six “complementarity determining regions” or “CDRs” present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as “framework” regions, show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see* “Sequences of Proteins of Immunological Interest,” Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987)).

[0042] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term “complementarity determining region” (“CDR”) to describe the non-contiguous antigen

combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth in the table below as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

	Kabat	Chothia
CDR-H1	31-35	26-32
CDR-H2	50-65	52-58
CDR-H3	95-102	95-102
CDR-L1	24-34	26-32
CDR-L2	50-56	50-52
CDR-L3	89-97	91-96

[0043] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of “Kabat numbering” to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, “Kabat numbering” refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequence of Proteins of Immunological Interest” (1983).

[0044] In addition to table above, the Kabat number system describes the CDR regions as follows: CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino

acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue); includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (*i.e.*, following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

[0045] Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be condrictoid in origin (*e.g.*, from sharks).

[0046] As used herein, the term “heavy chain constant region” includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0047] The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG₁ molecule and a hinge region derived from an IgG₃ molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG₁ molecule and, in part, from an IgG₃

molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG₁ molecule and, in part, from an IgG₄ molecule.

[0048] As used herein, the term “light chain constant region” includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

[0049] A “light chain-heavy chain pair” refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

[0050] As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term “VH domain” includes the amino terminal variable domain of an immunoglobulin heavy chain and the term “CH1 domain” includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0051] As used herein the term “CH2 domain” includes the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; *see* Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0052] As used herein, the term “hinge region” includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol* 161:4083 (1998)).

[0053] As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CK regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0054] As used herein, the term “chimeric antibody” will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0055] As used herein, “percent humanization” is calculated by determining the number of framework amino acid differences (*i.e.*, non-CDR difference) between the humanized domain and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

[0056] By “specifically binds” or “has specificity to,” it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to “specifically bind” to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term “specificity” is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody “A” may be deemed to have a higher specificity for a given epitope than antibody “B,” or antibody “A” may be said to bind to epitope “C” with a higher specificity than it has for related epitope “D.”

[0057] As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or

slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0058] By “subject” or “individual” or “animal” or “patient” or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

[0059] As used herein, phrases such as “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

Anti-SIRPα Antibodies

[0060] The present disclosure provides anti-SIRPα antibodies and fragments that have high affinity to both variants v1 and v2. Variant 1 (hSIRPαV1) is the dominant variant among Europeans, Africans, Ad mixed Americans, and South Asians. Variant 2 (hSIRPαV2) is the dominant variant among East Asians. Sequences of hSIRPαV1 and hSIRPαV2 differ within the extracellular Ig-like V-like (IgV) domain. The ability of the instantly disclosed antibodies and fragments to recognize both variants enables them to be effective among the widest patient population.

[0061] Moreover, the instant antibodies and fragments exhibited excellent binding affinity, potent induction of macrophage-mediated phagocytosis, and superior chemistry, manufacturing, and control (CMC) developability.

[0062] In accordance with one embodiment of the present disclosure, therefore, provided are antibodies and antigen-binding fragments thereof that are able to bind to both variants 1 and 2 of SIRPα. Example antibodies include those murine ones listed in **Table 1**, as well as humanized ones of **Tables 2-8**. Also included are those that include the same CDRs as

illustrated herein. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as those illustrated here, and those that compete with the instantly disclosed in binding to SIRP α .

[0063] In accordance with one embodiment of the present disclosure, provided is an antibody or fragment thereof that includes the heavy chain and light chain variable domains with the CDR regions disclosed herein, as well as their biological equivalents.

[0064] In one embodiment, the CDRs are those of 248G3F6, as exemplified in **Tables 2B and 2D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0065] One embodiment provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

[0066] One embodiment provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 21, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

[0067] One embodiment provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

[0068] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27.

[0069] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29.

[0070] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:27 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:29.

[0071] In one embodiment, the CDRs are those of 300A6A6, as exemplified in **Tables 3B and 3D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0072] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0073] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid

sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0074] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 37, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0075] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 38, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0076] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44.

[0077] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46.

[0078] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:43 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:45.

[0079] In one embodiment, the CDRs are those of 102A10F2, as exemplified in **Tables 4B and 4D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0080] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52.

[0081] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1,

CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 53, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52.

[0082] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52.

[0083] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60.

[0084] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6 and 61-62, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and 61-62.

[0085] In one embodiment, the CDRs are those of 62D2H6, as exemplified in **Tables 5B and 5D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 63 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 64 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 65 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 66 or a

variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 67 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 68 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0086] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and 69-72, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:7 and 69-72.

[0087] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:8 and 73-76, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and 73-76.

[0088] In one embodiment, the CDRs are those of 211F8E11, as exemplified in **Tables 6B and 6D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 77 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 78 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 79 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 80 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 81 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 82 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0089] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:9 and 83-86, or a peptide having

at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:9 and 83-86.

[0090] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:10 and 87-90, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 10 and 87-90.

[0091] In one embodiment, the CDRs are those of 217D11E5, as exemplified in **Tables 7B and 7D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 91 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 92 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 93 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 94 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 95 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 96 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0092] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:11 and 97-100, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:11 and 97-100.

[0093] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:12 and 101-102, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12 and 101-102.

[0094] In one embodiment, the CDRs are those of 234B7D5, as exemplified in **Tables 8B and 8D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 103 or a variant thereof having one, two, or three deletions, additions, substitutions or the

combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 104 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 105 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 106 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 107 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 108 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0095] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:13 and 109-112, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:13 and 109-112.

[0096] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:14 and 113-118, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 14 and 113-118.

[0097] The antibodies that contained these CDR regions, whether mouse, humanized or chimeric, had potent SIRP α binding and inhibitory activities. As shown in **Example 5**, certain residues within the CDR can be modified to retain or improve the property or reduce their potential to have post-translational modifications (PTMs). Such modified CDR can be referred to as affinity matured or de-risked CDRs.

[0098] Non-limiting examples of de-risked CDRs are provided in **Tables 2B, 3B and 4B**. Modified CDRs can include those having one, two or three amino acid addition, deletion and/or substitutions. In some embodiments, the substitutions can be conservative substitutions.

[0099] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains

(*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0100] Non-limiting examples of conservative amino acid substitutions are provided in the table below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Table A. Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Table B. Conservative Amino Acid Substitutions

For Amino Acid	Substitution With
Alanine	D-Ala, Gly, Aib, β -Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser

Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β -Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

[0101] It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0102] In certain embodiments, the antibody comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, an antibody of the disclosure may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0103] Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation,

metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

[0104] In some embodiments, the antibodies may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0105] The antibodies may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

[0106] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0107] The antibodies can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Techniques for conjugating various moieties to an antibody are well known, *see, e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.*, (eds.), Marcel Dekker, Inc., pp. 623- 53 (1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), Academic Press pp. 303-16 (1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* (52:119-58 (1982)).

Bi-functional Molecules and Combination Therapies

[0108] An important component of the innate immune system is macrophages. Macrophages inhibit tumor growth through phagocytosis of tumor cells. Tumor cells can evade surveillance by macrophages by upregulating a “don’t eat me” signal, CD47, on their cell surface. CD47 is ubiquitously expressed but is upregulated in various tumor types. By interacting with various ligands, CD47 has roles in the regulation of cell motility, adhesion, migration, and platelet activation. SIRP α is another member of the immunoglobulin superfamily and is primarily expressed on the surface of neurons and myeloid cells, including macrophages, granulocytes, monocytes, and dendritic cells. The CD47/SIRP α axis is a known major pathway for immune evasion by tumor cells.

[0109] Agents that target CD47 may initiate antibody opsonization, and activate antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) which aids tumor cell destruction. However, due to the ubiquitous expression of CD47 may lead to RBC toxicity and other hematological adverse effects due to the presence of the antigen on the blood cells. Molecules targeting either SIRP α or bispecific agents that target both SIRP α and CD47 can be designed to elicit the ADCC and ADCP effect by possessing functional Fc domains.

[0110] Since SIRP α is more restricted in its expression compared to CD47, these molecules should not have as many hematological events as those targeting only CD47. Therefore, anti-SIRP α antibodies can be safer than anti-CD47 antibodies. Another option is the development of bispecific agents that target both the CD47/SIRP α axis and another tumor antigen. These agents can either be bispecific antibodies, fusion proteins and combination therapies.

[0111] Examples of tumor antigens, in particular those that can trigger tumor opsonizing, include CD19, CD20, EGFR, HER2, CD3, CD16, PD1, PD-L1, LAG3, TIM3, CTLA4, VISTA, CSFR1, A2AR, CD73, CD39, CD40, CEA, HER2, VEGFR, TIGIT, claudin18.2, CD24, GPC3, IL13RA2, 4-1BB, CCR8, and CMET, without limitation.

[0112] Different format of bispecific antibodies are also provided. In some embodiments, each of the anti-SIRP α fragment and the second fragment each is independently selected from a Fab fragment, a single-chain variable fragment (scFv), or a single-domain antibody. In some embodiments, the bispecific antibody further includes a Fc fragment.

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0113] The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the antibodies, variants or derivatives thereof of the disclosure. The polynucleotides of the present disclosure may encode the entire heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules. Additionally, the polynucleotides of the present disclosure may encode portions of the heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules.

[0114] Methods of making antibodies are well known in the art and described herein. In certain embodiments, both the variable and constant regions of the antigen-binding polypeptides of the present disclosure are fully human. Fully human antibodies can be made using techniques described in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in U.S. patents: 6,150,584; 6,458,592; 6,420,140 which are incorporated by reference in their entireties.

Treatment Methods

[0115] As described herein, the antibodies, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0116] The present disclosure is further directed to antibody-based therapies which involve administering the antibodies of the disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, antibodies of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding antibodies of the disclosure (including variants and derivatives thereof as described herein).

[0117] The antibodies of the disclosure can also be used to treat or inhibit cancer. As provided above, SIRP α can be overexpressed in tumor cells, in particular gastric, pancreatic,

esophageal, ovarian, and lung tumors. Inhibition of SIRP α has been shown to be useful for treating the tumors.

[0118] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-express SIRP α .

[0119] Cellular therapies, such as chimeric antigen receptor (CAR) T-cell therapies, are also provided in the present disclosure. A suitable cell can be used, that is put in contact with an anti-SIRP α antibody of the present disclosure (or alternatively engineered to express an anti-SIRP α antibody of the present disclosure). Upon such contact or engineering, the cell can then be introduced to a cancer patient in need of a treatment. The cancer patient may have a cancer of any of the types as disclosed herein. The cell (e.g., T cell) can be, for instance, a tumor-infiltrating T lymphocyte, a CD4⁺ T cell, a CD8⁺ T cell, or the combination thereof, without limitation.

[0120] In some embodiments, the cell was isolated from the cancer patient him- or her-self. In some embodiments, the cell was provided by a donor or from a cell bank. When the cell is isolated from the cancer patient, undesired immune reactions can be minimized.

[0121] Non-limiting examples of cancers include bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer. In some embodiments, the cancer is one or more of gastric, pancreatic, esophageal, ovarian, and lung cancers.

[0122] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not

limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[0123] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated.

Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0124] Methods of administration of the antibodies, variants or include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray.

[0125] The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0126] Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0127] It may be desirable to administer the antigen-binding polypeptides or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0128] The amount of the antibodies of the disclosure which will be effective in the treatment, inhibition and prevention of an inflammatory, immune or malignant disease, disorder or condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0129] As a general proposition, the dosage administered to a patient of the antigen-binding polypeptides of the present disclosure is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight, between 0.1 mg/kg and 20 mg/kg of the patient's body weight, or 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent

administration is often possible. Further, the dosage and frequency of administration of antibodies of the disclosure may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0130] In an additional embodiment, the compositions of the disclosure are administered in combination with cytokines. Cytokines that may be administered with the compositions of the disclosure include, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, and TNF- α .

[0131] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Diagnostic Methods

[0132] Over-expression of SIRP α is observed in certain tumor samples, and patients having SIRP α -over-expressing cells are likely responsive to treatments with the anti-SIRP α antibodies of the present disclosure. Accordingly, the antibodies of the present disclosure can also be used for diagnostic and prognostic purposes.

[0133] A sample that preferably includes a cell can be obtained from a patient, which can be a cancer patient or a patient desiring diagnosis. The cell be a cell of a tumor tissue or a tumor block, a blood sample, a urine sample or any sample from the patient. Upon optional pre-treatment of the sample, the sample can be incubated with an antibody of the present disclosure under conditions allowing the antibody to interact with a SIRP α protein potentially present in the sample. Methods such as ELISA can be used, taking advantage of the anti-SIRP α antibody, to detect the presence of the SIRP α protein in the sample.

[0134] Presence of the SIRP α protein in the sample (optionally with the amount or concentration) can be used for diagnosis of cancer, as an indication that the patient is suitable for a treatment with the antibody, or as an indication that the patient has (or has not) responded to a cancer treatment. For a prognostic method, the detection can be done at once, twice or more, at certain stages, upon initiation of a cancer treatment to indicate the progress of the treatment.

Compositions

[0135] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an antibody, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

[0136] In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a “pharmaceutically acceptable carrier” will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0137] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified

form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0138] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0139] The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1: Generation of murine monoclonal antibodies against human SIRPα

[0140] The human SIRPα protein was used to immunize different strains of mice and hybridomas were generated accordingly. Eight fusions were made to generate sufficient number of hybridoma clones. SIRPα v1/v2 positive binders were selected and subcloned. Subsequently, *in vitro* binding and functional screening were carried out with about 30 purified antibodies and lead antibodies with highest binding affinity and strongest functional potency were identified. The lead antibodies were humanized.

[0141] The VH/VL sequences of the lead murine antibodies are provided in the table below.

Table 1. VH/VL sequence of the lead murine antibodies

Name	Sequence (CDRs are underlined)	SEQ ID NO:
248G3F6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE <u>DTYMH</u> WVKQRPDQGLEWIGR <u>IDPADGDTKYNPKFQD</u> KATITVDTSNTAYLQLSSLTSEDTAVYYCVRGN <u>YVNWGGQTTLTVSS</u>	1
248G3F6 VL	QIVLIQSPAIMASAPGERVTLT <u>CRASSSVSSSYLY</u> WYQQKPGSSPKLWIY <u>STSNLAS</u> GVPARFSGSGSGTSYSLTISSEAEADAASYFC <u>HOWSYSPRT</u> FG GGTKLEIK	2
300A6A6 VH	QVQLQQSGTELVKPGSSVKISCKASGYTFT <u>SNYIHW</u> IRQQPGNGLEWIGW <u>IYPGDGDTNYNQKFNKG</u> KATLTADKSSSTAYMQLSSLTSEDTAVYFCAINY <u>GGIWFAYWGQGITLVTVSS</u>	3
300A6A6 VL	DIQMTQSPSSMSASLGDRVTITC <u>QASQDIGNKLI</u> WFQQKPGKSPRLMIHY <u>VTNLPG</u> GVPLRFSGSRSGSDYSLTISSELESDMADYYC <u>LOYKQNP</u> LTFGS GTKLEIK	4
102A10F2 VH	QVTLKESGPGILQPSQTLSTCSFSGFSLN <u>TYDIGMG</u> WIRQPSGKGLEWL <u>AHIWWDREYNSALQS</u> RVITISKDTSNTQVFLKIASVDTADTATYYCVR <u>I</u> <u>DYFGSGQAWFTYWGQGITLVTVSA</u>	5
102A10F2 VL	EIVLTQSPPTMAASPGEKITITC <u>SSSSSTISSTYLHW</u> YQQKPGFSPKLLIS <u>GTSNLAS</u> GVPPRFSGSGSGTSYSLTIGTLEAEDVATYYC <u>QQGSRIPT</u> FG SGTKLEIK	6
62D2H6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK <u>DYMH</u> WVKQRTEQGLEWIGR <u>IDPEDGETKYAPKFOG</u> KATITADTSSNTAYLQLSSLTSEDTAVYYCSR <u>SW</u> <u>AYWGQGITTLTVSS</u>	7
62D2H6 VL	QIVLTQSPAIMASAPGEKVTLTCS <u>SASSSVSSSYLY</u> WYQQKPGSSPKLWIY <u>STSNLAS</u> GVPARFSGSGSGTSYSLTISSEAEADAASYFC <u>HOWSSYPRT</u> FG GGTKLEIK	8
211F8E11 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK <u>DTYMH</u> WVKQRPEQGLEWVGR <u>IDPANVNTIYDPKFOG</u> KATITADTSSNTAYLQLSSLTSEDTAVYYCAR <u>VG</u> <u>AYDGYDFDYWGQGITTLTVSS</u>	9
211F8E11 VL	DIVLTQSPASLAVSLGQRATISCRASEVDNYGN <u>SFMH</u> WYQQKPGPPKL LIY <u>RASNLES</u> GIPARFSGSGSRTDFTLTINPVEADDVATYYC <u>QQNNEDPL</u> <u>TFGAGTKLELK</u>	10
217D11E5 VH	EVQLQQSGPELVKPGASVKMSCKASGYTFT <u>SYVMH</u> WVKQKPGQGLEWIGY <u>INPYNDGTYNEKFKG</u> KATLTSDKSSSTAYMELSSLTSEDSAVYYCAR <u>SY</u> <u>YDYDGSFDYWGQGITTLTVSS</u>	11
217D11E5 VL	DIVMTQSHKFMSTSVGDRVSITC <u>KASQDVTTAVA</u> WYQQKSGQSPKLLIY <u>S</u> <u>ASYRYT</u> GDPRFTGSGSGTDFTFTISSVQAEDLAVYYC <u>QOHYSTPWT</u> FGG GTKLEIK	12
234B7D5 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE <u>DTYIHW</u> WVKQRPDQGLEWIGR <u>IDPADGDTKHNPKFHD</u> KATITVDTSNTAYLELSSLTSEDTAVYYCVRGN <u>YVNWGGQTTLTVSS</u>	13
234B7D5 VL	QIVLIQSPAIMASAPGERVTLT <u>CRASSSVTSSYLY</u> WYQQKPGSSPKLWIY <u>SASNLAS</u> GVPARFSGSGSGTSYSLTISSEAEADAASYFC <u>HOWSYSPRT</u> FG GGTKLEIK	14

Example 2. Cross-binding of SIRPα v1 and v2

[0142] This example measured the dose response of ELISA binding of mouse anti-SIRPα mAb to recombinant human SIRPα variant 1 and variant 2 protein (0.5 μg/ml@100 μl). Recombinant human SIRPα v1 or v2 protein (Biointron) was coated at 0.5 μg/ml in PBS onto microtiter plates for 2 h at RT. After coating of the antigen, the wells were blocked with PBS/0.05% Tween (PBST) with 1% BSA for 1 h at RT.

[0143] After washing of the wells with PBST, different concentrations of anti-SIRP α antibodies were added to the well and incubated for 1 h at RT. For detection of the binding antibodies, the HRP conjugated secondary antibodies against mouse Fc (Jackson Immuno Research) were added followed by the addition of fluorogenic substrates (Roche). Between all incubation steps, the wells of the plate were washed with PBST three times. Fluorescence was measured in a TECAN Spectrafluor plate reader.

[0144] The results are shown in **FIG. 1**. Both tested antibodies, 248G3F6 and 300A6A6, exhibited nanogram level affinity to both variants 1 and 2.

[0145] The binding kinetics assay of antibody to variant 1 was performed using Biacore 8K system through human antibody capture approach. The anti-mouse Fc IgG were immobilized on CM5 sensor chip according to the manufactory's instruction. The test antibody was injected and captured by the immobilized anti-human Fc IgG. Serial concentrations of antigen was individually injected, and the binding profile was recorded for each concentration antigen analyte, respectively.

[0146] The assay system was regenerated by injection of 10 mM Glycine-HCl pH 1.5 for 30 seconds. The running buffer was HBS-EP+ (10mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA and 0.05% P20). The assay temperature was 25 °C, and the association and dissociation time were 180 and 600 seconds, respectively. The Biacore data were fitted using Biacore K8 evaluation software 1.0 according to 1:1 binding model to calculate the association (k_a) and dissociation (k_d) rate constants as well as the equilibrium constant (K_D).

[0147] The results are shown in **FIG. 2**, and summarized in the table below. Both tested antibodies exhibited excellent binding affinity.

Sample	k_a (1/Ms)	k_d (1/s)	K_D (M)
248G3F6	1.87E+05	3.74E-04	2.00E-09
300A6A6	1.31E+05	1.48E-04	1.13E-09

Example 3. Competition with CD47

[0148] This example tested the ability of the anti-SIRP α antibodies to compete with CD47 in binding to SIRP α .

[0149] Recombinant CD47-Fc fusion protein (Acrobiosystems) was coated at 1 µg/ml in PBS onto microtiter plates for 16 hours at 4 °C. After blocking for 1 h with 1% BSA in PBST at RT, 1 µg/mL of SIRPα-His protein was added either in the absence or presence of different concentrations of anti-SIRPα antibodies at RT for 1 h. Plates were subsequently washed three times and incubated with an HRP-conjugated anti-His secondary antibody for 1 h at RT. After washing, the TMB solution was added to each well for 30 min and the reaction was stopped with 2M H₂SO₄, and OD was measured at 490 nm.

[0150] As shown in **FIG. 3**, both 248G3F6 and 300A6A6 potently and dose-dependently inhibited the binding of CD47 to SIRPα.

Example 4. Induction of macrophage mediated phagocytosis

[0151] This example tested the ability of the anti-SIRPα antibodies to induce macrophage mediated phagocytosis.

[0152] PBMCs were isolated from human blood, and the monocytes were differentiated into macrophages for 6 days. The monocyte derived macrophages (MDMs) were scraped and re-plated in 24-well dishes and allowed to adhere for 24 hours. The human tumor cell line Raji which endogenously expressed CD47 were transfected with human PD-L1 to overexpress human PD-L1 on the surface. This PD-L1 overexpressed Raji cells were chosen as target cells and labeled with 1 µM CFSE for 10 minutes, then added to MDMs at a ratio of 5:1 tumor cells per phagocyte.

[0153] Anti-SIRPα antibodies and anti-PD-L1 antibody were added in the culture system. After incubation for 3 hours, non-phagocytosed target cells were washed away with PBS and the remaining phagocytes were scraped off, stained with macrophage marker C76 antibody, and analyzed by flow cytometry. Phagocytosis was measured by gating on C76⁺ cells and then assessing the percent of CFSE⁺ cells.

[0154] The results of phagocytosis of PD-L1 expressing tumor cells by combo-treatment of anti-SIRPα antibody with anti-PD-L1 antibody are shown in **FIG. 4**. The combination of anti-PD-L1 antibody with either of the anti-SIRPα antibodies exhibited the highest phagocytosis (the two columns on the right).

Example 5. Humanization of the mouse mAbs

[0155] The murine antibody variable region genes were employed to create humanized mAbs. In the first step of this process, the amino acid sequences of the VH and VL of mAb were compared against the available database of human Ig gene sequences to find the overall best-matching human germline Ig gene sequences.

[0156] The amino acid sequences of the humanized antibody are provided below.

Humanized sequences

A. 248G3F6

Table 2A. Humanization of 248G3F6 – VH

Name	Sequence	SEQ ID NO:
248G3F6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE DTYMH WVKQRPDQGLEWIG R IDPADGDTKYNPKFQD KATITVDTSSNTAYLQLSSLTSEDIAVYYCVR GN YVN WGQGTITLVSS	1
V1 (CDR grafting)	QVQLVQSGAEVKKPGASVKVSKASGFNFED DTYMH WVRQAPGQGLEWMG R IDPADGDTKYNPKFQD RVMTMRDTSSTVYMESSLRSEDIAVYYCARG N YVN WGQGTITVTVSS	23
V2 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGFNFEDTYMHWVRQAPGQGLEWMG R IDPADGDTKYNPKFQDRVTMT VD TST NTA YMESSLRSEDIAVYYC VR GN YVNWGQGTITVTVSS	24
V3 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGFNFEDTYMHWVRQAPGQGLEWMG R IDPADGDTKYNPKFQDRVT I T VDTST NTA YMESSLRSEDIAVYYC VR GN YVNWGQGTITVTVSS	25
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGFNFEDTYMHWVRQAPGQGLEWMG R IDPA E GDTKYNPKFQDRVT I T VDTST NTA YMESSLRSEDIAVYYC VR GN YVNWGQGTITVTVSS	26
V5 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGFNFEDTYMHWVRQAPGQGLEWMG R IDPAD A DTKYNPKFQDRVT I T VDTST NTA YMESSLRSEDIAVYYC VR GN YVNWGQGTITVTVSS	27

Table 2B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DTYMH	15
CDR-H2	RIDPADGDTKYNPKFQD	16
CDR-H3	GNYVN	17
CDR-H2 (v4)	RIDPA E GDTKYNPKFQD	21
CDR-H2 (v5)	RIDPAD A DTKYNPKFQD	22

Table 2C. Humanization of 248G3F6 – VL

Name	Sequence	SEQ ID NO:
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248G3F6 VL	QIVLIQSPAIMSASPGERVLTLC <u>RASSSVSSSYLY</u> WYQQKPGSSPKLWIY <u>STSNLAS</u> GVPARFSGSGSGTSYSLTISSEAEADAASYFC <u>HQWYSYPRT</u> FG GGTKLEIK	2
V1 (CDR grafting)	EIVLTQSPGTLSSLSPGERATLSC <u>RASSSVSSSYLY</u> WYQQKPGQAPRLLIY <u>STSNLAS</u> GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC <u>HQWYSYPRT</u> FG GGTKVEIK	28
V2 (with back mutations)	EIVLTQSPGTLSSLSPGERATLSC <u>RASSSVSSSYLY</u> WYQQKPGQAPRLLIY STSNLASGIPDRFSGSGSGTDY <u>Y</u> TLTISRLEPED <u>A</u> AVY <u>F</u> CHQWYSYPRTFG GGTKVEIK	29

Table 2D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RASSSVSSSYLY	18
CDR-L2	STSNLAS	19
CDR-L3	HQWYSYPRT	20

Table 2E. Humanized antibodies

	VL	VL v1	VL v2
VH	HSP210-02-Chi		
VH v1		HSP210-02-hz11	HSP210-02-hz12
VH v2		HSP210-02-hz21	HSP210-02-hz22
VH v3		HSP210-02-hz31	HSP210-02-hz32
VH v4		HSP210-02-hz41	HSP210-02-hz42
VH v5		HSP210-02-hz51	<u>HSP210-02-hz52</u>

B. 300A6A6

Table 3A. Humanization of 300A6A6 – VH

Name	Sequence	SEQ ID NO:
300A6A6 VH	QVQLQQSGTELVKPGSSVKISCKASGYTFT <u>SNYI</u> HWIRQPGNGLEWIGW <u>IYPGDGDTNYNQKFNG</u> KATLTADKSSSTAYMQLSSLTSEDYAVYFC <u>AINY</u> <u>GGIWFAYWGQGLTVTVSS</u>	3
V1 (CDR grafting)	QVQLVQSGAEVKKPGSSVKVSKASGYTFT <u>SNYI</u> HWVRQAPGQGLEWMGW <u>IYPGDGDTNYNQKFNG</u> GRVTITADKSTSTAYMELSSLRSEDYAVYYC <u>ARNY</u> <u>GGIWFAYWGQGLTVTVSS</u>	39
V2 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDGDTNYNQKFNGRVTITADKSTSTAYMELSSLRSEDYAVYYC <u>AINY</u> GGIWFAYWGQGLTVTVSS	40
V3 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDGDTNYNQKFNGRVT <u>L</u> TADKSTSTAYMELSSLRSEDYAVYYC <u>AINY</u> GGIWFAYWGQGLTVTVSS	41
V4 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGE <u>G</u> DTNYNQKFNGRVT <u>L</u> TADKSTSTAYMELSSLRSEDYAVYYC <u>AINY</u> GGIWFAYWGQGLTVTVSS	42
V5 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGD <u>A</u> DTNYNQKFNGRVT <u>L</u> TADKSTSTAYMELSSLRSEDYAVYYC <u>AINY</u> GGIWFAYWGQGLTVTVSS	43
V6 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDGDTNYNQKF <u>Q</u> GRVT <u>L</u> TADKSTSTAYMELSSLRSEDYAVYYC <u>AINY</u> GGIWFAYWGQGLTVTVSS	44

Table 3B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	SNYIH	30
CDR-H2	WIYPGDGDTNYNQKFNG	31
CDR-H3	NYGGIWFAY	32
CDR-H2 (v4)	WIYPGE ~~G~~ DTNYNQKFNG	36
CDR-H2 (v5)	WIYPGDA ~~D~~ DTNYNQKFNG	37
CDR-H2 (v6)	WIYPGDGDTNYNQKFG ~~Q~~ G	38

Table 3C. Humanization of 300A6A6 – VL

Name	Sequence	SEQ ID NO:
300A6A6 VL	DIQMTQSPSSMSASLGDRVTITCQASQDIGNKLIWFQQKPGKSPRLMIHY VTNLP ~~G~~ GVPLRFSGSRSGSDYSLTISSESEDMADYYCLQYKQNP ~~LT~~ FGS GTKLEIK	4
V1 (CDR grafting)	DIQMTQSPSSLSASVGDRVTITCQASQDIGNKLIWFQQKPGKAPKLLIYY VTNLP ~~G~~ GVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQYKQNP ~~LT~~ FGQ GTKLEIK	45
V2 (with back mutations)	DIQMTQSPSSLSASVGDRVTITCQASQDIGNKLIWFQQKPGKAPKLLIHY VTNLP ~~G~~ GVPSRFSRSGSDY ~~T~~ LTISLQPEDFATYYCLQYKQNP ~~LT~~ FGQ GTKLEIK	46

Table 3D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	QASQDIGNKLI	33
CDR-L2	YVTNLP ~~G~~	34
CDR-L3	LQYKQNP ~~LT~~	35

Table 3E. Humanized antibodies

	VL	VL v1	VL v2
VH	HSP210-03-Chi		
VH v1		HSP210-03-hz11	HSP210-03-hz12
VH v2		HSP210-03-hz21	HSP210-03-hz22
VH v3		HSP210-03-hz31	HSP210-03-hz32
VH v4		HSP210-03-hz41	HSP210-03-hz42
VH v5		HSP210-03-hz51	HSP210-03-hz52
VH v6		HSP210-03-hz61	HSP210-03-hz62

C. 102A10F2**Table 4A. Humanization of 102A10F2 – VH**

Name	Sequence	SEQ ID NO:
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102A10F2 VH	QVTLKESGPGILQPSQTLSLTCSFSGFSLN TYDIGMG WIRQPSGKGLEWLA H IWWNDREYYNSALQSR VTISKDTSNTQVFLKIASVDTADTATYYCVR IDYFG SGQAWFTY WGQGTLLTVSSA	5
V1 (CDR grafting)	QLQLQESGPGLVKPSSETLSLTCTVSGFSLN TYDIGMG WIRQPPGKGLEWIGH IWWNDREYYNSALQSR VTISVDTSKNQFSLKLSSVTAADTAVYYCVR IDYFG SGQAWFTY WGQGTLLTVSS	55
V2 (with back mutations)	Q V QLQESGPGLVKPSSETLSLTCT F SGFSLNTYDIGMGWIRQPPGKGLEWIGH IWWNDREYYNSALQSRVTIS KD TSK TQV SLKLSSVTAADTAVYYCVRIDYFG SGQAWFTYWGQGTLLTVSS	56
V3 (with back mutations)	Q V QLQESGPGLVKPSSETLSLTCT F SGFSLNTYDIGMGWIRQPPGKGLEW I AH IWWNDREYYNSALQSRVTIS KD TSK TQV SLKLSSVTAADTAVYYCVRIDYFG SGQAWFTYWGQGTLLTVSS	57
V4 (with back mutations)	Q V QLQESGPGLVKPSSETLSLTCT F SGFSL S TYDIGMGWIRQPPGKGLEW I AH IWWNDREYYNSALQSRVTIS KD TSK TQV SLKLSSVTAADTAVYYCVRIDYFG SGQAWFTYWGQGTLLTVSS	58
V5 (with back mutations)	Q V QLQESGPGLVKPSSETLSLTCT F SGFSLNTYDIGMGWIRQPPGKGLEW I AH IWWNDREYY S SALQSRVTIS KD TSK TQV SLKLSSVTAADTAVYYCVRIDYFG SGQAWFTYWGQGTLLTVSS	59
V6 (with back mutations)	Q V QLQESGPGLVKPSSETLSLTCT F SGFSLNTYDIGMGWIRQPPGKGLEW I AH IWWNDREYYN P ALQSRVTIS KD TSK TQV SLKLSSVTAADTAVYYCVRIDYFG SGQAWFTYWGQGTLLTVSS	60

Table 4B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	TYDIGMG	47
CDR-H2	HIWWNDREYYNSALQS	48
CDR-H3	IDYFGSGQAWFTY	49
CDR-H2 (v5)	HIWWNDREYY S SALQS	53
CDR-H2 (v6)	HIWWNDREYYN P ALQS	54

Table 4C. Humanization of 102A10F2 – VL

Name	Sequence	SEQ ID NO:
102A10F2 VL	EIVLTQSPPTMAASPGKITTITC SSSSTISSTYLH WYQQKPGFSPKLLIS GTSN LASGVPPRFSGSGSGTISYSLTIIGTLEAEDVATYYC QQGSRIPFT FG SGTKLEIK	6
V1 (CDR grafting)	EIVLTQSPGTLSSLSPGERATLSC SSSSTISSTYLH WYQQKPGQAPRLLIY GTSN LASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC QQGSRIPFT FG QGKLEIK	61
V2 (with back mutations)	EIVLTQSPGTLSSLSPGERATLSC SSSSTISSTYLH WYQQKPGQAPRLLI S GTSNLAGIPDRFSGSGSGTD Y TLTISRLEPED V AVYYC QQGSRIPFT FG QGKLEIK	62

Table 4D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	SSSSTISSTYLH	50
CDR-L2	GTSNLAS	51
CDR-L3	QQGSRIPFT	52

Table 3E. Humanized antibodies

	VL	VL v1	VL v2
VH	HSP210-01-Chi		
VH v1		HSP210-01-hz11	HSP210-01-hz12
VH v2		HSP210-01-hz21	HSP210-01-hz22
VH v3		HSP210-01-hz31	HSP210-01-hz32
VH v4		HSP210-01-hz41	HSP210-01-hz42
VH v5		HSP210-01-hz51	HSP210-01-hz52
VH v6		HSP210-01-hz61	HSP210-01-hz62

D. 62D2H6

Table 5A. Humanization of 62D2H6 – VH

Name	Sequence	SEQ ID NO:
62D2H6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIKDYMHVVKQRTEQGLEWIGR IDPEDGETKYAPKFQGRVITADTSSNTAYLQLSSLTSEDVAVYYCSR AYWGQGTTITVSS	7
V1 (CDR grafting)	EVQLVQSGAEVKKPGATVKISCKVSGFNIKDYMHVVKQAPGKLEWMGR IDPEDGETKYAPKFQGRVITADTSTDYAMELSSLRSEDVAVYYCATSW AYWGQGTTITVSS	69
V2 (with back mutations)	EVQLVQSGAEVKKPGATVKISCKASGFNIKDYMHVVKQAPGKLEWMGR IDPEDGETKYAPKFQGRVITADTSTNTAYAMELSSLRSEDVAVYYCSR AYWGQGTTITVSS	70
V3 (chimeric 2)	QVQLVQSGAEVKKPGASVKVSKASGFNIKDYMHVVKQAPGKLEWMGR IDPEDGETKYAPKFQGRVITMTRDTSTSTVYMESSLRSEDVAVYYCAR AYWGQGTTITVSS	71
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGFNIKDYMHVVKQAPGKLEWMGR IDPEDGETKYAPKFQGRVITMTADTSTNTAYAMELSSLRSEDVAVYYCSR AYWGQGTTITVSS	72

Table 5B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DYYMH	63
CDR-H2	RIDPEDGETKYAPKFQG	64
CDR-H3	SWAY	65

Table 5C. Humanization of 62D2H6 – VL

Name	Sequence	SEQ ID NO:
62D2H6 VL	QIVLTQSPAIMSASPGEKVTLTCSASSSVSSSYLYWYQQKPGSSPKLWIYST SNLASGVPARFSGSGSGTSYSLTISSEAEADAASYFCHQWSSYPRTFGGGK LEIK	8
V1 (CDR grafting)	EIVLTQSPATLSLSPGERATLSCASSSVSSSYLYWYQQKPGQAPRLLIYST SNLASGIPARFSGSGSGTDFLTITISSEPEDFAVYYCHQWSSYPRTFGGGK VEIK	73
V2 (with back mutations)	EIVLTQSPATLSLSPGERATLSCASSSVSSSYLYWYQQKPGQAPRLLIYST SNLASGIPARFSGSGSGTDYTLTISSEPEDAAVYFCHQWSSYPRTFGGGK VEIK	74

V3 (chimeric 2)	EIV <u>MTQSPPTLSLSPGERV</u> TLSCSASSSVSSSYLYWYQQKPGQAPRLLIYST SNLASGIPARFSGSGSGTDFTLTISSL <u>Q</u> PEDFAVYYCHQWSSYPRTFGGGTK VEIK	75
V4 (with back mutations)	EIV <u>MTQSPPTLSLSPGERV</u> TLSCSASSSVSSSYLYWYQQKPGQAPRLLIYST SNLASGIPARFSGSGSGTD <u>Y</u> LTLTISSL <u>Q</u> PE <u>D</u> <u>A</u> V <u>Y</u> <u>F</u> CHQWSSYPRTFGGGTK VEIK	76

Table 5D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	SASSSVSSSYLY	66
CDR-L2	STSNLAS	67
CDR-L3	HQWSSYPRT	68

Table 5E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4
VH	Chimeric				
VH v1		hz11			
VH v2			hz22		hz24
VH v3				hz33	
VH v4			hz42		hz44

E. 211F8E11**Table 6A. Humanization of 211F8E11 – VH**

Name	Sequence	SEQ ID NO:
211F8E11 VH	EVQLVQSGAELVKPGASVKLSCTASGFNIKDTYMHVVKQRPEQGLEWVGR <u>IDPANVNTIYDPKFQ</u> GKATITADTSSNTAYLQLSSLTSEDIAVYYCARV <u>G</u> <u>AYDGYDFDY</u> WGQGITLVSS	9
V1 (CDR grafting)	EVQLVQSGAEVKKPGATVKISCKVSGFNIKDTYMHVVKQAPGKGLEWMGR <u>IDPANVNTIYDPKFQ</u> GRVTITADTSTDYAMELSSLRSEDIAVYYCATV <u>G</u> <u>AYDGYDFDY</u> WGQGITTVSS	83
V2 (with back mutations)	EVQLVQSGAEVKKPGATVKISCKASGFNIKDTYMHVVKQAPGKGLEWMGR IDPANVNTIYDPKFQGRVTITADTSTNTAYAMELSSLRSEDIAVYYCARVG AYDGYDFDYWGQGITTVSS	84
V3 (chimeric 2)	QVQLVQSGAEVKKPGASVKVSKASGFNIKDTYMHVVRQAPGQGLEWMGR IDPANVNTIYDPKFQGRVTMTTRDTSTSTVYMESSLRSEDIAVYYCARVG AYDGYDFDYWGQGITTVSS	85
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGFNIKDTYMHVVRQAPGQGLEWMGR IDPANVNTIYDPKFQGRVTMTADTSTNTAYAMELSSLRSEDIAVYYCARVG AYDGYDFDYWGQGITTVSS	86

Table 6B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DTYMH	77
CDR-H2	RIDPANVNTIYDPKFQ	78
CDR-H3	VGAYDGYDFDY	79

Table 6C. Humanization of 211F8E11 – VL

Name	Sequence	SEQ ID NO:
211F8E11 VL	DIVLTQSPASLAVSLGQRATISCRASESDNYGNSFMHWYQQKPGQPPKLLI YRASNLESGIPARFSGSGSRDFTLTINPVEADDVATYYCQNNEDPLTFGA GTKLELK	10
V1 (CDR grafting)	DIVMTQSPDSLAVSLGERATINCRASESDNYGNSFMHWYQQKPGQPPKLLI YRASNLESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNNEDPLTFGQ GTKLEIK	87
V2 (with back mutations)	DIVLTQSPDSLAVSLGERATINCRASESDNYGNSFMHWYQQKPGQPPKLLI YRASNLESGVPDRFSGSGSRDFTLTISLQAEDVAVYYCQNNEDPLTFGQ GTKLEIK	88
V3 (chimeric 2)	DIQMTQSPSSLSASVGDRVITTCRASESDNYGNSFMHWYQQKPGKVPKLLI YRASNLESGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQNNEDPLTFGQ GTKLEIK	89
V4 (with back mutations)	DIQLTQSPSSLSASVGDRVITTCRASESDNYGNSFMHWYQQKPGKVPKLLI YRASNLESGVPSRFSGSGSRDFTLTISLQPEDVATYYCQNNEDPLTFGQ GTKLEIK	90

Table 6D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	PASESDNYGNSFMH	80
CDR-L2	RASNLES	81
CDR-L3	QNNEDPLI	82

Table 6E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4
VH	Chimeric				
VH v1		hz11			
VH v2			hz22		hz24
VH v3				hz33	
VH v4			hz42		hz44

F. 217D11E5**Table 7A. Humanization of 217D11E5 – VH**

Name	Sequence	SEQ ID NO:
217D11E5 VH	EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGGLEWIGY INPYNDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARSY YDYDGSFDYWGQGITLVSS	11
V1 (CDR grafting)	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYVMHWVRQAPGQRLEWMGY INPYNDGTKYNEKFKGRVTITRDTASTAYMELSSLRSEDATVYYCARSY YDYDGSFDYWGQGITLVSS	97
V2 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYVMHWVRQAPGQRLEWMGY INPYNDGTKYNEKFKGRVTITSDKASTAYMELSSLRSEDATVYYCARSY YDYDGSFDYWGQGITLVSS	98

V3 (chimeric 2)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGY INPYNDGTKYNEKFKGRVTMTTRDTSTSTVYMESSLRSEDTAVYYCARSY YDYDGSFDYWGQGTTITVSS	99
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGY INPYNDGTKYNEKFKGRVTMTSDKSTSTAYMESSLRSEDTAVYYCARSY YDYDGSFDYWGQGTTITVSS	100

Table 7B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	SYVMH	91
CDR-H2	YINPYNDGTKYNEKFKG	92
CDR-H3	SYDYDGSFDY	93

Table 7C. Humanization of 217D11E5 – VL

Name	Sequence	SEQ ID NO:
217D11E5 VL	DIVMTQSHKFMSTSVGDRVSITC <u>KASQDVTTAVA</u> WYQQKSGQSPKLLIY <u>SAS</u> <u>YRYT</u> GDPDRFTGSGSGTDFFTISSVQAEDLAVYYC <u>QQHYSTPWT</u> FGGGTKL EIK	12
V1 (CDR grafting)	DIQMTQSPSSLSASVGDRVTITC <u>KASQDVTTAVA</u> WYQQKPGKAPKLLIY <u>SAS</u> <u>YRYT</u> GVPSRFSGSGSGTDFFTISSLPEDVATYYC <u>QQHYSTPWT</u> FGGGTKV EIK	101
V2 (chimeric 2)	DIQMTQSPSSLSASVGDRVTITC <u>KASQDVTTAVA</u> WYQQKPGKVPKLLIY <u>SAS</u> YRYTGVPSRFSGSGSGTDFLTISSLPEDVATYYC <u>QQHYSTPWT</u> FGGGTKV EIK	102

Table 7D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	KASQDVTTAVA	94
CDR-L2	SASYRYT	95
CDR-L3	QQHYSTPWT	96

Table 7E. Humanized antibodies

	VL	VL v1	VL v2
VH	Chimeric		
VH v1		hz11	
VH v2		hz21	hz22
VH v3			
VH v4		hz41	hz42

G. 234B7D5**Table 8A. Humanization of 234B7D5 – VH**

Name	Sequence	SEQ ID NO:
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234B7D5 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE DTYIH HWVKQRPDQGLEWIG GRID PADGDTKHNPKFHD KATVTVDTSSNTAYLELSSLTSEDTAVYYCVR GNVYNW GQGTTITVSS	13
V1 (CDR grafting)	EVQLVQSGAEVKKPGATVKISCKVSGFNFE DTYIH HWVQQAPGKGLEWM GRID PADGDTKHNPKFHD RVITITADTSTDTAYMELSSLRSEDTAVYYCAT GNVYNW GQGTTITVSS	109
V2 (with back mutations)	EVQLVQSGAEVKKPGATVKISCKASGFNFEDTYIHWVQQAPGKGLEWMGRID PADGDTKHNPKFHDRVITITVDTSTNTAYMELSSLRSEDTAVYYCVRGNVYNW GQGTTITVSS	110
V3 (chimeric 2)	QVQLVQSGAEVKKPGASVKVSKASGFNFEDTYIHWVRQAPGQGLEWMGRID PADGDTKHNPKFHDRVIMTRDTSTSTVYMELSSLRSEDTAVYYCARGNVYNW GQGTTITVSS	111
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGFNFEDTYIHWVRQAPGQGLEWMGRID PADGDTKHNPKFHDRVIMTVDTSTNTAYMELSSLRSEDTAVYYCVRGNVYNW GQGTTITVSS	112

Table 8B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DTYIH	103
CDR-H2	RIDPADGDTKHNPKFHD	104
CDR-H3	GNVYN	105

Table 7C. Humanization of 234B7D5 – VL

Name	Sequence	SEQ ID NO:
234B7D5 VL	QIVLIQSPAIMASASPGERVTLT CRASSSVTSSYLY WYQQKPGSSPKLWIY SA SNLAS GVPARFSGSGSGTSYSLTISSEVAEDAASVFC HQWYSYPRT FGGGTK LEIK	14
V1 (CDR grafting)	EIVLTQSPATLSLSPGERATLS CRASSSVTSSYLY WYQQKPGQAPRLLIY SA SNLAS GIPARFSGSGSGTDFTLTISSELPEDFAVYYC HQWYSYPRT FGGGTK VEIK	113
V2 (with back mutations)	EIVLTQSPATLSLSPGERATLS CRASSSVTSSYLY WYQQKPGQAPRLLIY SA SNLAS GIPARFSGSGSGTDYTLTISSELPEDAADVFC HQWYSYPRT FGGGTK VEIK	114
V3 (chimeric 2)	EIVLTQSPGTLTLSPGERATLS CRASSSVTSSYLY WYQQKPGQAPRLLIY SA SNLAS GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC HQWYSYPRT FGGGTK VEIK	115
V4 (with back mutations)	EIVLTQSPGTLTLSPGERATLS CRASSSVTSSYLY WYQQKPGQAPRLLIY SA SNLAS GIPDRFSGSGSGTDYTLTISRLEPEDAAVFC HQWYSYPRT FGGGTK VEIK	116
V5 (chimeric 3)	EIVMTQSPPTLSLSPGERVTLS CRASSSVTSSYLY WYQQKPGQAPRLLIY SA SNLAS GIPARFSGSGSGTDFTLTISLQPEDFAVYYC HQWYSYPRT FGGGTK VEIK	117
V6 (with back mutations)	EIVMTQSPPTLSLSPGERVTLS CRASSSVTSSYLY WYQQKPGQAPRLLIY SA SNLAS GIPARFSGSGSGTDYTLTISLQPEDAAVFC HQWYSYPRT FGGGTK VEIK	118

Table 8D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RASSSVTSSYLY	106
CDR-L2	SASNLAS	107
CDR-L3	HQWYSYPPT	108

Table 8E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4	VL v5	VL v6
VH	Chimeric						
VH v1		hz11					
VH v2			hz22		hz24		hz26
VH v3				hz33			
VH v4			hz42		hz44		hz46

Example 6. Testing of Humanized Antibodies

[0157] This example tested some of the humanized antibodies for the ability to block interactions between SIRP α and CD47.

[0158] Recombinant CD47-Fc fusion protein (Acrobiosystems) was coated at 1 μ g/ml in PBS onto microtiter plates for 16 hours at 4 °C. After blocking for 1 h with 1% BSA in PBST at RT, 1 μ g/mL of SIRP α -His protein was added either in the absence or presence of different concentrations of the anti-SIRP α antibodies at RT for 1 h. Plates were subsequently washed three times and incubated with an HRP-conjugated anti-His secondary antibody for 1 h at RT. After washing, the TMB solution was added to each well for 30 min and the reaction was stopped with 2M H₂SO₄, and OD was measured at 490 nm.

[0159] All of the antibodies listed in **Tables 2E** (248G3F6), **3E** (300A6A6), and **4E** (102A10F2) were tested and exhibited high IC₅₀ (**Table 9**).

Table 9. Activities of humanized antibodies to block SIRP α interaction with CD47

	Antibody	IC ₅₀ (nM)
248G3F6	02-chi	0.14
	02-hz22	0.092
	02-hz32	0.11
	02-hz42	0.12
	02-hz52	0.11
300A6A6	03-chi	0.14
	03-hz22	0.16
	03-hz32	0.145
	03-hz42	0.13
	03-hz52	0.13
102A10F2	01-hz22	0.21
	01-hz32	0.16
	01-hz52	0.23
	01-hz61	0.21

	01-hz62	0.16
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Example 7. Increase of macrophage mediated phagocytosis of tumor cells

[0160] This example tested some of the humanized antibodies for their ability to increase macrophage mediated phagocytosis of tumor cells.

[0161] PBMCs were isolated from human blood, and monocytes were differentiated into macrophages using a standard protocol. The monocyte derived macrophages (MDMs) were scraped and re-plated in 24-well dishes and allowed to adhere for 24 hrs. The human tumor cell line Raji that endogenously expressed CD47 were selected as target cells and labeled with 1uM CFSE for 10 mins, then added to MDMs at a ratio of 5:1 tumor cells per phagocyte and different concentrations of anti-SIRP α antibodies was added at the indicated concentrations. After 3hr incubation, non-phagocytosed target cells were washed away with PBS and the remaining phagocytes were scraped off, stained with C76 antibody, and analyzed by flow cytometry. Phagocytosis was measured by gating on C76⁺ cells and then assessing the percentage of CFSE⁺ cells.

[0162] The results are presented in **FIG. 5**. Out of the tested antibodies, 02-hz52 (248G3F6) and 03-hz51 (300A6A6) exhibited the highest activities, and all others showed excellent activities as well.

Example 8. Binding affinity to SIRP α v1 and v2

[0163] Humanized antibodies 02-hz52 (248G3F6) and 03-hz51 (300A6A6) were tested for their binding affinities to SIRP α v1 and v2 in this example.

[0164] The binding kinetics assay of antibody to antigen was performed using Biacore 8K system through human antibody capture approach. The anti-mouse Fc IgG were immobilized on CM5 sensor chip according to the manufactory's instruction. The test antibody was injected and captured by the immobilized anti-human Fc IgG. And then serial concentrations of human SIRP α v1 or SIRP α v2 protein were individually injected, and the binding profile was recorded for each concentration antigen analyte, respectively. The assay system was regenerated by injection of 10 mM Glycine-HCl pH 1.5 for 30 seconds. The running buffer was HBS-EP+ (10mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA and 0.05% P20). The assay temperature was 25 °C, and the association and dissociation time were 180 and 600

seconds, respectively. The Biacore data were fitted using Biacore K8 evaluation software 1.0 according to 1:1 binding model to calculate the association (k_a) and dissociation (k_d) rate constants as well as the equilibrium constant (K_D).

[0165] The testing results are shown in **Table 10A-B**.

Table 10A. Binding affinity against SIRP α v1

Sample	k_a (1/Ms)	k_d (1/s)	K_D (M)
02-hz52	7.04E+05	2.98E-04	4.23E-10
03-hz51	8.05E+05	8.04E-04	9.99E-10

Table 10B. Binding affinity against SIRP α v2

Sample	k_a (1/Ms)	k_d (1/s)	K_D (M)
02-hz52	3.33E+05	2.47E-03	7.40E-09
03-hz51	3.31E+05	1.00E-03	3.03E-09

Example 9. Developability Studies

[0166] Antibodies 02-hz52 (248G3F6) and 03-hz51 (300A6A6), fused to human IgG4 heavy chain constant regions and kappa light chain constant regions, were tested for their developability. Both antibodies do not contain free cysteines. The testing results showed that both antibodies can be purified to reach clinical grade purities, have suitable T_m , acid/base peak ratios, hydrophobicity, and pI. The results are presented in **Table 11**.

[0167] Table 10A. Binding affinity against SIRP α v1

Sample	Aggregates	SEC (% monomer)	Yield (mg/L)	pI	Acidic peak	Main peak	Basic peak	T_m pH7.5 (°C)	HIC (NH ₄) ₂ SO ₄ (M)
02-hz52	Not detected	99.2	>10	6.9	35.5	57.2	7.2	65/71	1.02
03-hz51	Not detected	97.91	>10	7.9	34.5	62.3	3.2	65/74	0.96

Example 10. *In vivo* Testing

[0168] This example tested the efficacy of humanized antibody 03-hz51 (300A6A6) in mice, alone or in combination with rituximab.

[0169] Raji-Luc cells resuspended in PBS were inoculated into the tail vein of B-NDG-hSIRP α humanized mice at a concentration of 1×10^5 cells/0.2 mL and a volume of 0.2 mL/head. A small animal imaging device was used to measure the tumor imaging signal value on the third day of inoculation. When the average imaging signal intensity reached 1.35×10^6 p/sec, the animals were divided into groups according to the tumor imaging signal

value and animal weight, and allocated evenly in 4 experiments There were 6 mice in each experimental group.

[0170] Rituximab exhibited a significant inhibitory effect on Raji-Luc lymphoma at a dose level of 10 mg/kg, with no clinically adverse symptoms. 03-hz51 in combination with rituximab (10 mg/kg+0.1 mg/kg) showed a significant inhibitory effect on Raji-Luc lymphoma. The data show that 03-hz51 synergized with rituximab in the complete inhibition of tumor growth in the Raji lymphoma tumor model.

* * *

[0171] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0172] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. An antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein:

- (a) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20;
- (b) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35;
- (c) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52;
- (d) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 63, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 64, the CDRH3 comprises

the amino acid sequence of SEQ ID NO: 65, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 66, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 67, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 68;

(e) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 77, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 78, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 79, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 80, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 81, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 82;

(f) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 91, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 92, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 93, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 94, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 95, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 96; or

(g) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 103, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 104, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 105, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 106, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 107, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 108.

2. An antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and

wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

3. The antibody or fragment thereof of claim 2, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27.

4. The antibody or fragment thereof of claim 2 or 3, wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29.

5. The antibody or fragment thereof of claim 2, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:27 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:29.

6. An antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence

of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

7. The antibody or fragment thereof of claim 6, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44.

8. The antibody or fragment thereof of claim 6 or 7, wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46.

9. The antibody or fragment thereof of claim 6, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:43 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:45.

10. An antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRP α) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52.

11. The antibody or fragment thereof of claim 10, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60.
12. The antibody or fragment thereof of claim 10 or 11, wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6 and 61-62, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and 61-62.
13. The antibody or fragment thereof of any one of claims 1-12, which can bind to SIRP α variant 1 and variant 2.
14. The antibody or fragment thereof of any one of claims 1-13, which is humanized.
15. The antibody or fragment thereof of any one of claims 1-24, which further has a binding specificity to a second target protein.
16. A composition comprising the antibody or fragment thereof of any one of claims 1-15 and a pharmaceutically acceptable carrier.
17. The composition of claim 16, further comprising a second antibody having specificity to a tumor antigen.
18. The composition of claim 17, wherein the second antibody is a tumor-opsonizing antibody.

19. A method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of any one of claims 1-15.
20. The method of claim 19, wherein the cancer is selected from the group consisting of bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.
21. The method of claim 19, further comprising administering to the patient a second antibody targeting a protein that triggers tumor opsonizing.
22. The method of claim 21, wherein the protein is selected from the group consisting of CD19, CD20, EGFR, HER2, CD3, CD16, PD1, PD-L1, LAG3, TIM3, CTLA4, VISTA, CSFR1, A2AR, CD73, CD39, CD40, CEA, HER2, VEGFR, TIGIT, claudin18.2, CD24, GPC3, IL13RA2, 4-1BB, CCR8, and CMET.