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- (71) **Applicant** (for all designated States except US): **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th Floor, Oakland, California 94607-5200 (US).
- (72) **Inventors; and**
- (75) **Inventors/Applicants** (for US only): **ROSEN, Steven D.** [US/US]; 828 Clayton Street, San Francisco, California 94117 (US). **SINGER, Mark S.** [US/US]; 1915 Grant Street, Berkeley, California 94703 (US). **LEMJABBAR-ALAOUI, Hassan** [US/US]; 575 El Capitan Drive, Danville, California 94526 (US).
- (74) **Agent:** **BORDEN, Paula A.**; Bozicevic, Field & Francis LLP, 1900 University Avenue, Suite 200, East Palo Alto, California 94303 (US).

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(54) **Title:** ANTIBODY SPECIFIC FOR SULFATASE-2 AND METHODS OF USE THEREOF

(57) **Abstract:** The present disclosure provides antibodies specific for an epitope present on a sulfatase-2 polypeptide. The antibodies are useful in various treatment, diagnostic, and monitoring applications, which are also provided.



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ANTIBODY SPECIFIC FOR SULFATASE-2 AND METHODS OF USE THEREOF**CROSS-REFERENCE**

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/438,883, filed February 2, 2011, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. P01-AI905319 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Heparan sulfate (HS) proteoglycans (HSPGs) bind to multiple growth factors/morphogens and regulate their signaling. 6-*O*-sulfation of glucosamine within HS chains are removed by enzymatic action of sulfatase-1 (Sulf-1) and sulfatase-2 (Sulf-2). Sulf-1 and Sulf-2 are extracellular neutral-pH sulfatases. Sulf-2 has been implicated, based on its overexpression, in various cancers, including breast cancer, pancreatic cancer, hepatocellular carcinoma, gastric cancer, head & neck cancer, skin cancer, multiple myeloma, central nervous system neoplasms, non-small cell lung cancer (lung adenocarcinoma, and lung squamous cell carcinoma), and kidney cancer. In four cancers (non-small cell lung cancer, pancreatic cancer, hepatocellular carcinoma, and glioblastoma, there is direct evidence that Sulf-2 is a driver of carcinogenesis.

[0004] There is a need in the art for reducing cancer cell proliferation and tumor growth.

Literature

[0005] Lemjabbar-Alaoui et al. (2010) *Oncogene* 29:635; U.S. Patent Publication No. 2003/0147875; Morimoto-Tomita et al. (2002) *J. Biol. Chem.* 277:49175; Morimoto-Tomita et al. (2005) *Neoplasia* 7:1001; Lai et al. (2008) *Hepatol.* 47:1211; Nawroth et al. (2007) *PLoS One* 2:e392; Johansson et al. (2005) *Oncogene* 24:3896-3905; Chen et al. (2010) *Hepatol.* 52:1957.

SUMMARY OF THE INVENTION

[0006] The present disclosure provides antibodies specific for sulfatase-2. The antibodies are useful in various treatment, diagnostic, and monitoring applications, which are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Figure 1 depicts reaction of anti-Sulf-2 antibodies with native Sulf-2.

[0008] Figure 2 depicts a western blot with 8G1 mAb, 5D5 mAb, or mIgG₁, with the 75 kDa subunit of recombinant Sulf-2 and native Sulf-2.

[0009] Figure 3 depicts the effect of anti-Sulf-2 antibodies on growth of H292 cells *in vitro*.

- [0010] Figure 4 depicts the effect of anti-Sulf-2 antibodies, compared to Sulf-2 knockdown, on growth of H292 cells *in vitro*.
- [0011] Figure 5 depicts the effect of anti-Sulf-2 antibodies on cell migration of H292 cells *in vitro*.
- [0012] Figure 6 depicts the effect of anti-Sulf-2 antibodies on colony formation by H292 cells in agarose.
- [0013] Figures 7A and 7B depict the effect of monoclonal antibody (mAb) 8G1 on growth of H292 cells *in vivo* in nude mice.
- [0014] Figure 8 provides an amino acid sequence of sulfatase-2 (Sulf-2).
- [0015] Figure 9 provides: an amino acid sequence of the 8G1 antibody heavy chain variable region; and an amino acid sequence of the 8G1 antibody light chain variable region. Complementarity determining regions (CDRs) are underlined.
- [0016] Figure 10 provides: an amino acid sequence of the 5D5 antibody heavy chain variable region; and an amino acid sequence of the 5D5 antibody light chain variable region. CDRs are underlined.
- [0017] Figure 11 provides: an amino acid sequence of the 2E8 antibody heavy chain variable region; and an amino acid sequence of the 2E8 antibody light chain variable region. CDRs are underlined.

DEFINITIONS

- [0018] The terms "antibodies" and "immunoglobulin" include antibodies or immunoglobulins of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to, Fab, Fv, scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies, and fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein. The antibodies may be detectably labeled, *e.g.*, with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like. The antibodies may be further conjugated to other moieties, such as members of specific binding pairs, *e.g.*, biotin (member of biotin-avidin specific binding pair), and the like. The antibodies may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like. Also encompassed by the term are Fab', Fv, F(ab')₂, and or other antibody fragments that retain specific binding to antigen, and monoclonal antibodies. An antibody may be monovalent or bivalent.
- [0019] "Antibody fragments" comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

[0020] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0021] The "Fab" fragment also contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0022] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

[0023] "Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see *Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)*.

[0024] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and *Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)*.

[0025] As used herein, the term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as a dissociation constant (Kd). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold

greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1000-fold greater, or more, than the affinity of an antibody for unrelated amino acid sequences. Affinity of an antibody to a target protein can be, for example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM) or more. As used herein, the term “avidity” refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms “immunoreactive” and “preferentially binds” are used interchangeably herein with respect to antibodies and/or antigen-binding fragments.

[0026] The term “binding” refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. A subject anti-sulfatase-2 binds specifically to an epitope within a sulfatase-2 polypeptide. Non-specific binding would refer to binding with an affinity of less than about 10^{-7} M, e.g., binding with an affinity of 10^{-6} M, 10^{-5} M, 10^{-4} M, etc.

[0027] As used herein, the term “CDR” or “complementarity determining region” is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat et al., J. Biol. Chem. 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, “Sequences of proteins of immunological interest” (1991); by Chothia et al., J. Mol. Biol. 196:901-917 (1987); MacCallum et al., J. Mol. Biol. 262:732-745 (1996); and in “Antibody Engineering” (Springer Lab Manuals), Roland Kontermann and Stefan Duebel (2001), Chapter 21: “Protein sequence analysis and structure analysis of antibody variable domains” by Andrew Martin, pages 422-442, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of any of these definitions to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by the above cited Kabat, Chothia, and MacCallum references are set forth below in Table 1 as a comparison.

Table 1: CDR Definitions

	Kabat¹	Chothia²	MacCallum³
V _H CDR1	31-35	26-32	30-35
V _H CDR2	50-65	53-55	47-58
V _H CDR3	95-102	96-101	93-101
V _L CDR1	24-34	26-32	30-36
V _L CDR2	50-56	50-52	46-55
V _L CDR3	89-97	91-96	89-96

¹ Residue numbering follows the nomenclature of Kabat et al., *supra*

² Residue numbering follows the nomenclature of Chothia et al., *supra*

³ Residue numbering follows the nomenclature of MacCallum et al., *supra*

[0028] As used herein, the term “framework” when used in reference to an antibody variable region is intended to mean all amino acid residues outside the CDR regions within the variable region of an antibody. A variable region framework is generally a discontinuous amino acid sequence between about 100-120 amino acids in length but is intended to reference only those amino acids outside of the CDRs. As used herein, the term “framework region” is intended to mean each domain of the framework that is separated by the CDRs.

[0029] An “isolated” antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the antibody will be purified (1) to greater than 90%, greater than 95%, or greater than 98%, by weight of antibody as determined by the Lowry method, for example, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or nonreducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. In some instances, isolated antibody will be prepared by at least one purification step.

[0030] As used herein, the terms “treatment,” “treating,” and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment,” as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0031] The terms “individual,” “subject,” “host,” and “patient,” used interchangeably herein, refer to a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc.

[0032] A “therapeutically effective amount” or “efficacious amount” refers to the amount of a subject anti-Sulf-2 antibody that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The “therapeutically effective amount” will vary depending on the anti-Sulf-2 antibody, the disease and its severity and the age, weight, etc., of the subject to be treated.

[0033] A “biological sample” encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells

derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as polynucleotides. The term “biological sample” encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[0034] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0035] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0036] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0037] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an antibody” includes a plurality of such antibodies and reference to “the anti-Sulf-2 antibody” includes reference to one or more anti-Sulf-2 antibodies and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0038] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0039] The present disclosure provides antibodies specific for a sulfatase-2 (Sulf-2) polypeptide. The antibodies are useful in various treatment, diagnostic, and monitoring applications, which are also provided.

ANTIBODIES

[0040] A subject antibody specifically binds a Sulf-2 polypeptide. In some embodiments, an epitope of a Sulf-2 polypeptide can be formed by a polypeptide having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 50 amino acids (aa) to about 100 aa, from about 100 aa to about 200 aa, from about 200 aa to about 300 aa, from 300 aa to about 400 aa, from about 400 aa to about 500 aa, from about 500 aa to about 600 aa, from about 600 aa to about 700 aa, from about 700 aa to about 800 aa, or from about 800 aa to 846 aa, of amino acids 25-870 of the sequence depicted in Figure 8 (SEQ ID NO:1).

[0041] In some embodiments, a subject anti-Sulf-2 antibody recognizes an epitope within the 75 kDa subunit of Sulf-2 (amino acid residues 25-538).

[0042] Amino acid sequences of Sulf-2 polypeptides are known in the art. See, e.g., Morimoto-Tomita et al. (2002) *J. Biol. Chem.* 277:49175; and sequences provided under the following GenBank accession numbers: 1) GenBank Accession No. NP_001155313 (*Homo sapiens* Sulf-2); 2) GenBank Accession No. NP_001030099.1 (*Rattus norvegicus* Sulf-2); 3) GenBank Accession No. NP_001041555.1 (*Canis lupus familiaris* Sulf-2); 4) GenBank Accession No. BAC38279.1 (*Mus musculus* Sulf-2); 5) GenBank Accession No. NP_001088414.1 (*Xenopus laevis* Sulf-2); and 6) GenBank Accession No. NP_001003833.2 (*Danio rerio* Sulf-2).

[0043] A subject antibody exhibits high affinity binding to Sulf-2. For example, a subject antibody binds to Sulf-2 with an affinity of at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, at least about 10^{-10} M, at least about 10^{-11} M, or at least about 10^{-12} M, or greater than 10^{-12} M. A subject antibody binds to an epitope present on a Sulf-2 polypeptide with an affinity of from about 10^{-7} M to about 10^{-8} M, from about 10^{-8} M to about 10^{-9} M, from about 10^{-9} M to about 10^{-10} M, from about 10^{-10} M to about 10^{-11} M, or from about 10^{-11} M to about 10^{-12} M, or greater than 10^{-12} M.

[0044] A subject anti-Sulf-2 antibody does not substantially cross-react with a polypeptide other than a Sulf-2 polypeptide. For example, a subject anti-Sulf-2 antibody does not substantially bind to a Sulf-1 polypeptide. As noted in Morimoto-Tomita et al. (2002) *supra*, Sulf-1 polypeptides from mouse and human share only about 63%-65% amino acid sequence identity with Sulf-2 polypeptides from mouse and human. A subject antibody exhibits substantially no binding to any epitopes formed by amino acids within a Sulf-1 polypeptide. Amino acid sequences of Sulf-1 polypeptides are known in the art. See, e.g., Morimoto-Tomita et al. (2002) *supra*; and GenBank Accession No. NP_001121676.1. Any binding of a subject antibody to an epitope formed by amino acids within a Sulf-1 polypeptide is generally non-specific binding of a substantially lower affinity than the specific binding of the

antibody to the epitope on a Sulf-2 polypeptide. A substantially lower affinity is generally at least a two fold, three fold, five fold, 10 fold, 50 fold, 100 fold, 500 fold, or 1000 fold lower affinity.

[0045] An enzymatically active Sulf-2 polypeptide has endoglucosamine-6-sulfatase activity, e.g., an enzymatically active Sulf-2 polypeptide selectively removes 6-O-sulfate groups from heparan sulfate. Heparan sulfate is present in a number of proteoglycans, called heparan sulfate proteoglycans (HSPGs). HSPGs can function as coreceptors for numerous heparin-binding growth factors and cytokines and are involved in cell signaling.

[0046] In some instances, a subject anti-Sulf-2 antibody can reduce binding of a Sulf-2 polypeptide to a heparan sulfate proteoglycan ligand. For example, a subject antibody can binding of a Sulf-2 polypeptide to a heparan sulfate proteoglycan ligand by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the degree of binding between the Sulf-2 polypeptide and the heparan sulfate proteoglycan ligand in the absence of the antibody.

[0047] In some instances, a subject anti-Sulf-2 antibody can reduce enzymatic activity of a Sulf-2 polypeptide. For example, in some instances, a subject anti-Sulf-2 antibody can reduce enzymatic activity of a Sulf-2 polypeptide by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the enzymatic activity of the Sulf-2 polypeptide in the absence of a subject anti-Sulf-2 antibody.

[0048] In some instances, a subject anti-Sulf-2 antibody can reduce cancer cell growth *in vitro* and/or *in vivo*, e.g., a subject anti-Sulf-2 antibody can reduce growth of a Sulf-2-overexpressing cancer cell. For example, in some instances, a subject anti-Sulf-2 antibody can reduce cancer cell growth *in vitro* and/or *in vivo* by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the rate of growth of the cancer cell in the absence of the anti-Sulf-2 antibody.

[0049] In some instances, a subject anti-Sulf-2 antibody can reduce one or more of growth, anchorage-independent growth, and migration of a cancer cell *in vitro*. For example, in some instances, a subject anti-Sulf-2 antibody can reduce one or more of growth, anchorage-independent growth, and migration of a cancer cell *in vitro* by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the growth rate, anchorage-independent growth, or migration of the cancer cell *in vitro* in the absence of the anti-Sulf-2 antibody.

[0050] In some cases, a subject anti-Sulf-2 antibody can reduce tumor size and/or cancer cell number *in vivo*, e.g., in some cases, a subject anti-Sulf-2 antibody can reduce tumor size and/or cancer cell

number *in vivo* by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the tumor size and/or cancer cell number in the absence of the anti-Sulf-2 antibody.

[0051] The term "antibody" refers to a protein comprising one or more (e.g., one or two) heavy chain variable regions (VH) and/or one or more (e.g., one or two) light chain variable regions (VL), or subfragments thereof capable of binding an epitope. The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions (CDR)", interspersed with regions that are more conserved, termed "framework regions (FR)". The extent of the FR and CDRs has been precisely defined (see, Kabat, et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia et al. (1987) J. Mol. Biol. 196: 901-917). A VH can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Similarly, a VL can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0052] The VH or VL chain of an antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy and two light chains, wherein the heavy and light chains are interconnected by, for example, disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable regions of the heavy and light chains comprise binding regions that interact with antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues and factors, including various cells of the immune system and the first component of the complement system. The term "antibody" includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM and subtypes thereof. In some embodiments, a subject antibody is an IgG isotype.

[0053] As used herein the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes; and numerous immunoglobulin variable region genes. Full-length immunoglobulin light chains (about 25 kD or 214 amino acids) are encoded by a variable region gene at the N-terminus (about 110 amino acids) and a kappa or lambda constant region at the C-terminus. Full-length immunoglobulin heavy chains (about 50 kD or 446 amino acids) are encoded by a variable region gene at the N-terminus (about 116 amino acids) and one of the other aforementioned constant region genes at the C-terminus, e.g. gamma (encoding about 330 amino acids). In some embodiments, a subject antibody comprises full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain.

[0054] In some embodiments, a subject antibody does not comprise a full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain, and instead comprises antigen-binding fragments of a full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain. In some embodiments, the antigen-binding fragments are contained on separate polypeptide chains; in other embodiments, the antigen-binding fragments are contained within a single polypeptide chain. The term "antigen-binding fragment" refers to one or more fragments of a full-length antibody that are capable of specifically binding to a Sulf-2 polypeptide, as described above. Examples of binding fragments include (i) a Fab fragment (a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment (a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment (consisting of the VH and CH1 domains); (iv) a Fv fragment (consisting of the VH and VL domains of a single arm of an antibody); (v) a dAb fragment (consisting of the VH domain); (vi) an isolated CDR; (vii) a single chain Fv (scFv) (consisting of the VH and VL domains of a single arm of an antibody joined by a synthetic linker using recombinant means such that the VH and VL domains pair to form a monovalent molecule); (viii) diabodies (consisting of two scFvs in which the VH and VL domains are joined such that they do not pair to form a monovalent molecule; the VH of each one of the scFv pairs with the VL domain of the other scFv to form a bivalent molecule); (ix) bi-specific antibodies (consisting of at least two antigen binding regions, each region binding a different epitope). In some embodiments, a subject antibody fragment is a Fab fragment. In some embodiments, a subject antibody fragment is a single-chain antibody (scFv).

[0055] In some embodiments, a subject antibody is a recombinant or modified antibody, e.g., a chimeric, humanized, deimmunized or an *in vitro* generated antibody. The term "recombinant" or "modified" antibody as used herein is intended to include all antibodies that are prepared, expressed, created, or isolated by recombinant means, such as (i) antibodies expressed using a recombinant expression vector transfected into a host cell; (ii) antibodies isolated from a recombinant, combinatorial antibody library; (iii) antibodies isolated from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes; or (iv) antibodies prepared, expressed, created, or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant antibodies include humanized, CDR grafted, chimeric, deimmunized, and *in vitro* generated antibodies; and can optionally include constant regions derived from human germline immunoglobulin sequences.

[0056] In some embodiments, a subject antibody competes for binding to a Sulf-2 polypeptide with an antibody that comprises:

[0057] (i) a V_LCDR1 comprising an amino acid sequence of SEQ ID NO:11, NO:17, or SEQ ID NO:23;

[0058] (ii) a V_LCDR2 comprising an amino acid sequence of SEQ ID NO:12, SEQ ID NO:18, or SEQ ID NO:24;

- [0059] (iii) a V_LCDR3 comprising an amino acid sequence of SEQ ID NO:13, SEQ ID NO:19, or SEQ ID NO:25;
- [0060] (iv) a V_HCDR1 comprising an amino acid sequence of SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:20;
- [0061] (v) a V_HCDR2 comprising an amino acid sequence of SEQ ID NO:9, SEQ ID NO:15, or SEQ ID NO:21; and
- [0062] (vi) a V_HCDR3 comprising an amino acid sequence of SEQ ID NO:10, SEQ ID NO:16, or SEQ ID NO:22.
- [0063] In some embodiments, a subject antibody competes for binding to a Sulf-2 polypeptide with an antibody that comprises V_L and V_H CDRs of 8G1, e.g., a subject antibody competes for binding to a Sulf-2 polypeptide with an antibody that comprises a V_L CDR1 comprising SEQ ID NO:11; a V_L CDR2 comprising SEQ ID NO:12; a V_L CDR3 comprising SEQ ID NO:13; a V_H CDR1 comprising SEQ ID NO:8, a V_H CDR2 comprising SEQ ID NO:9; and a V_H CDR3 comprising SEQ ID NO:10.
- [0064] In some embodiments, a subject antibody comprises a V_L CDR1 comprising SEQ ID NO:11; a V_L CDR2 comprising SEQ ID NO:12; a V_L CDR3 comprising SEQ ID NO:13; a V_H CDR1 comprising SEQ ID NO:8, a V_H CDR2 comprising SEQ ID NO:9; and a V_H CDR3 comprising SEQ ID NO:10.
- [0065] In some embodiments, a subject antibody competes for binding to a Sulf-2 polypeptide with an antibody that comprises V_L and V_H CDRs of 5D5, e.g., a subject antibody competes for binding to a Sulf-2 polypeptide with an antibody that comprises a V_L CDR1 comprising SEQ ID NO:17; a V_L CDR2 comprising SEQ ID NO:18; a V_L CDR3 comprising SEQ ID NO:19; a V_H CDR1 comprising SEQ ID NO:14, a V_H CDR2 comprising SEQ ID NO:15; and a V_H CDR3 comprising SEQ ID NO:16.
- [0066] In some embodiments, a subject antibody comprises a V_L CDR1 comprising SEQ ID NO:17; a V_L CDR2 comprising SEQ ID NO:18; a V_L CDR3 comprising SEQ ID NO:19; a V_H CDR1 comprising SEQ ID NO:14, a V_H CDR2 comprising SEQ ID NO:15; and a V_H CDR3 comprising SEQ ID NO:16.
- [0067] In some embodiments, a subject antibody competes for binding to a Sulf-2 polypeptide with an antibody that comprises V_L and V_H CDRs of 2E8, e.g., a subject antibody competes for binding to a Sulf-2 polypeptide with an antibody that comprises a V_L CDR1 comprising SEQ ID NO:23; a V_L CDR2 comprising SEQ ID NO:24; a V_L CDR3 comprising SEQ ID NO:25; a V_H CDR1 comprising SEQ ID NO:20, a V_H CDR2 comprising SEQ ID NO:21; and a V_H CDR3 comprising SEQ ID NO:22.
- [0068] In some embodiments, a subject antibody comprises a V_L CDR1 comprising SEQ ID NO:23; a V_L CDR2 comprising SEQ ID NO:24; a V_L CDR3 comprising SEQ ID NO:25; a V_H CDR1 comprising SEQ ID NO:20, a V_H CDR2 comprising SEQ ID NO:21; and a V_H CDR3 comprising SEQ ID NO:22.

[0069] In some embodiments, a subject antibody comprises: a variable domain comprising: a) a heavy chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of the anti-Sulf-2 antibody designated 8G1; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the 8G1 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the 8G1 antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the 8G1 antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the 8G1 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the 8G1 antibody wherein the antibody specifically binds a Sulf-2 polypeptide.

[0070] In certain embodiments, an antibody comprising: a) a variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of an 8G1 antibody; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the 8G1 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the 8G1 antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the 8G1 antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the 8G1 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the 8G1 antibody; or b) a variant of the variable domain of part a) that is otherwise identical to the variable domain of part a) except for a number of (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) amino acid substitutions in the CDR regions, where the antibody specifically binds a Sulf-2 polypeptide.

[0071] In some embodiments, a subject anti-Sulf-2 antibody comprises: a) a light chain region comprising: i) one, two, or three complementarity determining regions (CDRs) from the 8G1 light chain variable region sequence; and ii) a light chain framework region, e.g., a framework region from a human immunoglobulin light chain; and b) a heavy chain region comprising: i) one, two, or three CDRs from the 8G1 heavy chain variable region sequence; and ii) a heavy chain framework region, e.g., a framework region from a human immunoglobulin heavy chain.

[0072] In some embodiments, a subject antibody comprises: a variable domain comprising: a) a heavy chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of the anti-Sulf-2 antibody designated 5D5; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the 5D5 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the 5D5 antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the 5D5 antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the 5D5 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the 5D5 antibody, wherein the antibody specifically binds a Sulf-2 polypeptide.

[0073] In certain embodiments, an antibody comprising: a) a variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of a 5D5 antibody; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the 5D5 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the 5D5 antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the 5D5 antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the 5D5 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the 5D5 antibody; or b) a variant of the variable domain of part a) that is otherwise identical to the variable domain of part a) except for a number of (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) amino acid substitutions in the CDR regions, where the antibody specifically binds a Sulf-2 polypeptide.

[0074] In some embodiments, a subject anti-Sulf-2 antibody comprises: a) a light chain region comprising: i) one, two, or three complementarity determining regions (CDRs) from the 5D5 light chain variable region sequence; and ii) a light chain framework region, e.g., a framework region from a human immunoglobulin light chain; and b) a heavy chain region comprising: i) one, two, or three CDRs from the 5D5 heavy chain variable region sequence; and ii) a heavy chain framework region, e.g., a framework region from a human immunoglobulin heavy chain.

[0075] In some embodiments, a subject antibody comprises: a variable domain comprising: a) a heavy chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of the anti-Sulf-2 antibody designated 2E8; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the 2E8 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the 2E8 antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the 2E8 antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the 2E8 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the 2E8 antibody wherein the antibody specifically binds a Sulf-2 polypeptide.

[0076] In certain embodiments, an antibody comprising: a) a variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of a 2E8 antibody; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the 2E8 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the 2E8 antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the 2E8 antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the 2E8 antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the 2E8 antibody; or b) a variant of the variable domain of part a) that is otherwise identical to the

variable domain of part a) except for a number of (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) amino acid substitutions in the CDR regions, where the antibody specifically binds a Sulf-2 polypeptide.

[0077] In some embodiments, a subject anti-Sulf-2 antibody comprises: a) a light chain region comprising: i) one, two, or three complementarity determining regions (CDRs) from the 2E8 light chain variable region sequence; and ii) a light chain framework region, e.g., a framework region from a human immunoglobulin light chain; and b) a heavy chain region comprising: i) one, two, or three CDRs from the 2E8 heavy chain variable region sequence; and ii) a heavy chain framework region, e.g., a framework region from a human immunoglobulin heavy chain.

[0078] A subject antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence depicted in Figure 9 and set forth in SEQ ID NO:2. A subject antibody can comprise a heavy chain variable region comprising one, two, or three of the heavy chain complementarity determining regions (CDRs) having an amino acid sequence selected from one or more of SEQ ID NOs:8, 9, and 10.

[0079] In some embodiments, a subject antibody comprises a heavy chain variable region comprising one, two, or three of the heavy chain CDRs having an amino acid sequence selected from one or more of SEQ ID NOs:8, 9, and 10; and FR regions that are human sequences (e.g., encoded by human heavy chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a heavy chain variable region that comprises, in order from N-terminus to C-terminus: a human heavy chain FR1; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:8; a human heavy chain FR2; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:9; a human heavy chain FR3; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:10; and a human heavy chain FR4.

[0080] A subject antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence depicted in Figure 9 and set forth in SEQ ID NO:3. A subject antibody can comprise a light chain variable region comprising one, two, or three of the light chain CDRs having the amino acid sequence set forth in SEQ ID NOs:11, 12, and 13.

[0081] In some embodiments, a subject antibody comprises a light chain variable region comprising one, two, or three of the light chain CDRs having a polypeptide sequence selected from one or more of SEQ ID NOs:11, 12, and 13; and FR regions that are human sequences (e.g., encoded by human light chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a light chain variable region that comprises, in order from N-terminus to C-terminus: a human light chain FR1; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:11; a human light chain FR2; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:12; a human light chain FR3; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:13; and a human light chain FR4.

[0082] In some embodiments, a subject antibody comprises 8G1 heavy chain CDRs and 8G1 light chain CDRs in a single polypeptide chain, e.g., in some embodiments, a subject antibody is a scFv. In some embodiments, a subject antibody comprises, in order from N-terminus to C-terminus: a first amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:8; a second amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:9; a third amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:10; a fourth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR1 comprising the amino acid sequence set forth in SEQ ID:11; a fifth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:12; a sixth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:13; and a seventh amino acid sequence of from about 5 amino acids to about 25 amino acids in length.

[0083] A subject antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence depicted in Figure 10 and set forth in SEQ ID NO:4. A subject antibody can comprise a heavy chain variable region comprising one, two, or three of the heavy chain complementarity determining regions (CDRs) having an amino acid sequence selected from one or more of SEQ ID NOs:14, 15, and 16.

[0084] In some embodiments, a subject antibody comprises a heavy chain variable region comprising one, two, or three of the heavy chain CDRs having an amino acid sequence selected from one or more of SEQ ID NOs:14, 15, and 16; and FR regions that are human sequences (e.g., encoded by human heavy chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a heavy chain variable region that comprises, in order from N-terminus to C-terminus: a human heavy chain FR1; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:14; a human heavy chain FR2; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:15; a human heavy chain FR3; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:16; and a human heavy chain FR4.

[0085] A subject antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence depicted in Figure 10 and set forth in SEQ ID NO:5. A subject antibody can comprise a light chain variable region comprising one, two, or three of the light chain CDRs having the amino acid sequence set forth in SEQ ID NOs:17, 18, and 19.

[0086] In some embodiments, a subject antibody comprises a light chain variable region comprising one, two, or three of the light chain CDRs having a polypeptide sequence selected from one or more of SEQ ID NOs:17, 18, and 19; and FR regions that are human sequences (e.g., encoded by human

light chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a light chain variable region that comprises, in order from N-terminus to C-terminus: a human light chain FR1; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:17; a human light chain FR2; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:18; a human light chain FR3; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:19; and a human light chain FR4.

[0087] In some embodiments, a subject antibody comprises 5D5 heavy chain CDRs and 5D5 light chain CDRs in a single polypeptide chain, e.g., in some embodiments, a subject antibody is a scFv. In some embodiments, a subject antibody comprises, in order from N-terminus to C-terminus: a first amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:14; a second amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:15; a third amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:16; a fourth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR1 comprising the amino acid sequence set forth in SEQ ID:17; a fifth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:18; a sixth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:19; and a seventh amino acid sequence of from about 5 amino acids to about 25 amino acids in length.

[0088] A subject antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence depicted in Figure 11 and set forth in SEQ ID NO:6. A subject antibody can comprise a heavy chain variable region comprising one, two, or three of the heavy chain complementarity determining regions (CDRs) having an amino acid sequence selected from one or more of SEQ ID NOs:20, 21, and 22.

[0089] In some embodiments, a subject antibody comprises a heavy chain variable region comprising one, two, or three of the heavy chain CDRs having an amino acid sequence selected from one or more of SEQ ID NOs:20, 21, and 22; and FR regions that are human sequences (e.g., encoded by human heavy chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a heavy chain variable region that comprises, in order from N-terminus to C-terminus: a human heavy chain FR1; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:20; a human heavy chain FR2; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:21; a human heavy chain FR3; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:22; and a human heavy chain FR4.

[0090] A subject antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence depicted in Figure 11 and set forth in SEQ ID NO:7. A subject antibody can comprise a light chain variable region comprising one, two, or three of the light chain CDRs having the amino acid sequence set forth in SEQ ID NOs:23, 24, and 25.

[0091] In some embodiments, a subject antibody comprises a light chain variable region comprising one, two, or three of the light chain CDRs having a polypeptide sequence selected from one or more of SEQ ID NOs: 23, 24, and 25; and FR regions that are human sequences (e.g., encoded by human light chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a light chain variable region that comprises, in order from N-terminus to C-terminus: a human light chain FR1; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23; a human light chain FR2; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24; a human light chain FR3; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:25; and a human light chain FR4.

[0092] In some embodiments, a subject antibody comprises 2E8 heavy chain CDRs and 2E8 light chain CDRs in a single polypeptide chain, e.g., in some embodiments, a subject antibody is a scFv. In some embodiments, a subject antibody comprises, in order from N-terminus to C-terminus: a first amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:20; a second amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:21; a third amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:22; a fourth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23; a fifth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24; a sixth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:25; and a seventh amino acid sequence of from about 5 amino acids to about 25 amino acids in length.

[0093] In some embodiments, a subject antibody comprises, in order from N-terminus to C-terminus: a light chain FR1 region; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:11; a light chain FR2 region; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:12; a light chain FR3 region; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:13; optionally a light chain FR4 region; a linker region; optionally a heavy chain FR1 region; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:8; a heavy chain FR2 region; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:9; a heavy chain FR3 region; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:10; and a heavy chain FR4 region. In

some of these embodiments, each of the FR regions is a human FR region. The linker region can be from about 5 amino acids to about 50 amino acids in length, e.g., from about 5 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, or from about 45 aa to about 50 aa in length.

[0094] In some embodiments, a subject antibody comprises, in order from N-terminus to C-terminus: a light chain FR1 region; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:17; a light chain FR2 region; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:18; a light chain FR3 region; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:19; optionally a light chain FR4 region; a linker region; optionally a heavy chain FR1 region; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:14; a heavy chain FR2 region; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:15; a heavy chain FR3 region; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:16; and a heavy chain FR4 region. In some of these embodiments, each of the FR regions is a human FR region. The linker region can be from about 5 amino acids to about 50 amino acids in length, e.g., from about 5 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, or from about 45 aa to about 50 aa in length.

[0095] In some embodiments, a subject antibody comprises, in order from N-terminus to C-terminus: a light chain FR1 region; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23; a light chain FR2 region; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24; a light chain FR3 region; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:25; optionally a light chain FR4 region; a linker region; optionally a heavy chain FR1 region; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:20; a heavy chain FR2 region; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:21; a heavy chain FR3 region; a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:22; and a heavy chain FR4 region. In some of these embodiments, each of the FR regions is a human FR region. The linker region can be from about 5 amino acids to about 50 amino acids in length, e.g., from about 5 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, or from about 45 aa to about 50 aa in length.

[0096] Linkers suitable for use a subject antibody include “flexible linkers”. If present, the linker molecules are generally of sufficient length to permit some flexible movement between linked regions. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to polypeptides may be used in light of this disclosure.

[0097] Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[0098] Exemplary flexible linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, GSGGS_n (SEQ ID NO: 26) and GGGS_n (SEQ ID NO: 27), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are of interest since both of these amino acids are relatively unstructured, and therefore may serve as a neutral tether between components. Glycine polymers are of particular interest since glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary flexible linkers include, but are not limited to, GGSG (SEQ ID NO:28), GSGGG (SEQ ID NO:29), GSGSG (SEQ ID NO:30), GSGGG (SEQ ID NO:31), GGGSG (SEQ ID NO:32), GSSSG (SEQ ID NO:33), and the like. The ordinarily skilled artisan will recognize that design of a peptide conjugated to any elements described above can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

[0099] In some embodiments, a subject antibody is "humanized." The term "humanized antibody" refers to an antibody comprising at least one chain comprising variable region framework residues substantially from a human antibody chain (referred to as the acceptor immunoglobulin or antibody) and at least one CDR substantially from a mouse antibody, (referred to as the donor immunoglobulin or antibody). See, Queen et al., *Proc. Natl. Acad. Sci. USA* 86:10029 10033 (1989), U.S. Pat. No. 5,530,101, U.S. Pat. No. 5,585,089, U.S. Pat. No. 5,693,761, WO 90/07861, and U.S. Pat. No. 5,225,539. The constant region(s), if present, can also be substantially or entirely from a human immunoglobulin. In some embodiments, a subject antibody comprises one or more 8G1, 5D5, or 2E8 CDRs and one or more FR regions from a human antibody. Methods of making humanized antibodies are known in the art. See, e.g., U.S. Patent No. 7,256,273.

[00100] The substitution of mouse CDRs into a human variable domain framework can result in retention of their correct spatial orientation where, e.g., the human variable domain framework adopts the same or similar conformation to the mouse variable framework from which the CDRs originated. This can be achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of

several human antibodies. See Kettleborough et al., Protein Engineering 4:773 (1991); Kolbinger et al., Protein Engineering 6:971 (1993).

[00101] Having identified the complementarity determining regions of the murine donor immunoglobulin and appropriate human acceptor immunoglobulins, the next step is to determine which, if any, residues from these components should be substituted to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a human-anti-mouse-antibody (HAMA) response in humans. Art-recognized methods of determining immune response can be performed to monitor a HAMA response in a particular patient or during clinical trials. Patients administered humanized antibodies can be given an immunogenicity assessment at the beginning and throughout the administration of said therapy. The HAMA response is measured, for example, by detecting antibodies to the humanized therapeutic reagent, in serum samples from the patient using a method known to one in the art, including surface plasmon resonance technology (BIAcore) and/or solid-phase ELISA analysis. In many embodiments, a subject humanized antibody does not substantially elicit a HAMA response in a human subject.

[00102] Certain amino acids from the human variable region framework residues are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. The unnatural juxtaposition of murine CDR regions with human variable framework region can result in unnatural conformational restraints, which, unless corrected by substitution of certain amino acid residues, lead to loss of binding affinity.

[00103] The selection of amino acid residues for substitution can be determined, in part, by computer modeling. Computer hardware and software for producing three-dimensional images of immunoglobulin molecules are known in the art. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three-dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting points for construction of the molecular model. Chains or domains sharing at least 50% sequence identity are selected for modeling, and preferably those sharing at least 60%, 70%, 80%, or 90% sequence identity, or more than 90% identity (e.g., 95% identity, 98% identity, or 99% identity) are selected for modeling. The solved starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by energy minimization and by verifying that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

[00104] CDR and framework regions are as defined by Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md., 1987 and 1991); or as defined

by Martin in "Antibody Engineering" (Springer Lab Manuals), Roland Kontermann and Stefan Duebel (2001), Chapter 21: "Protein sequence analysis and structure analysis of antibody variable domains" by Andrew Martin, pages 422-442. An alternative structural definition has been proposed by Chothia et al., J. Mol. Biol. 196:901 (1987); Nature 342:878 (1989); and J. Mol. Biol. 186:651 (1989) (collectively referred to as "Chothia"). When framework residues, as defined by Kabat, supra, constitute structural loop residues as defined by Chothia, supra, or Martin, supra, the amino acids present in the mouse antibody may be selected for substitution into the humanized antibody. Residues which are "adjacent to a CDR region" include amino acid residues in positions immediately adjacent to one or more of the CDRs in the primary sequence of the humanized immunoglobulin chain, for example, in positions immediately adjacent to a CDR as defined by Kabat, or a CDR as defined by Chothia (See e.g., Chothia and Lesk JMB 196:901 (1987)), or a CDR as defined by Martin, supra. These amino acids are particularly likely to interact with the amino acids in the CDRs and, if chosen from the acceptor, to distort the donor CDRs and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit et al., Science, 233:747 (1986)) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

[00105] In some embodiments, a subject antibody comprises scFv multimers. For example, in some embodiments, a subject antibody is an scFv dimer (e.g., comprises two tandem scFv (scFv₂)), an scFv trimer (e.g., comprises three tandem scFv (scFv₃)), an scFv tetramer (e.g., comprises four tandem scFv (scFv₄)), or is a multimer of more than four scFv (e.g., in tandem). The scFv monomers can be linked in tandem via linkers of from about 2 amino acids to about 10 amino acids in length, e.g., 2 aa, 3 aa, 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, or 10 aa in length. Suitable linkers include, e.g., (Gly)_x, where x is an integer from 2 to 10. Other suitable linkers are those discussed above. In some embodiments, each of the scFv monomers in a subject scFv multimer is humanized, as described above.

[00106] In some embodiments, a subject antibody comprises a constant region of an immunoglobulin (e.g., an Fc region). The Fc region, if present, can be a human Fc region. If constant regions are present, the antibody can contain both light chain and heavy chain constant regions. Suitable heavy chain constant region include CH1, hinge, CH2, CH3, and CH4 regions. The antibodies described herein include antibodies having all types of constant regions, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. An example of a suitable heavy chain Fc region is a human isotype IgG1 Fc. Light chain constant regions can be lambda or kappa. A subject antibody (e.g., a subject humanized antibody) can comprise sequences from more than one class or isotype. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab' F(ab')₂, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

[00107] In some embodiments, a subject antibody comprises a free thiol (-SH) group at the carboxyl terminus, where the free thiol group can be used to attach the antibody to a second polypeptide (e.g., another antibody, including a subject antibody), a scaffold, a carrier, etc.

[00108] In some embodiments, a subject antibody comprises one or more non-naturally occurring amino acids. In some embodiments, the non-naturally encoded amino acid comprises a carbonyl group, an acetyl group, an aminooxy group, a hydrazine group, a hydrazide group, a semicarbazide group, an azide group, or an alkyne group. See, e.g., U.S. Patent No. 7,632,924 for suitable non-naturally occurring amino acids. Inclusion of a non-naturally occurring amino acid can provide for linkage to a polymer, a second polypeptide, a scaffold, etc. For example, a subject antibody linked to a water-soluble polymer can be made by reacting a water-soluble polymer (e.g., PEG) that comprises a carbonyl group to a subject antibody that comprises a non-naturally encoded amino acid that comprises an aminooxy, hydrazine, hydrazide or semicarbazide group. As another example, a subject antibody linked to a water-soluble polymer can be made by reacting a subject antibody that comprises an alkyne-containing amino acid with a water-soluble polymer (e.g., PEG) that comprises an azide moiety; in some embodiments, the azide or alkyne group is linked to the PEG molecule through an amide linkage. A "non-naturally encoded amino acid" refers to an amino acid that is not one of the 20 common amino acids or pyrrolysine or selenocysteine. Other terms that may be used synonymously with the term "non-naturally encoded amino acid" are "non-natural amino acid," "unnatural amino acid," "non-naturally-occurring amino acid," and variously hyphenated and non-hyphenated versions thereof. The term "non-naturally encoded amino acid" also includes, but is not limited to, amino acids that occur by modification (e.g. post-translational modifications) of a naturally encoded amino acid (including but not limited to, the 20 common amino acids or pyrrolysine and selenocysteine) but are not themselves naturally incorporated into a growing polypeptide chain by the translation complex. Examples of such non-naturally-occurring amino acids include, but are not limited to, N-acetylglucosaminyl-L-serine, N-acetylglucosaminyl-L-threonine, and O-phosphotyrosine.

[00109] In some embodiments, a subject antibody is linked (e.g., covalently linked) to a polymer (e.g., a polymer other than a polypeptide). Suitable polymers include, e.g., biocompatible polymers, and water-soluble biocompatible polymers. Suitable polymers include synthetic polymers and naturally-occurring polymers. Suitable polymers include, e.g., substituted or unsubstituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymers or branched or unbranched polysaccharides, e.g. a homo- or hetero-polysaccharide. Suitable polymers include, e.g., ethylene vinyl alcohol copolymer (commonly known by the generic name EVOH or by the trade name EVAL); polybutylmethacrylate; poly(hydroxyvalerate); poly(L-lactic acid); polycaprolactone; poly(lactide-co-glycolide); poly(hydroxybutyrate); poly(hydroxybutyrate-co-valerate); polydioxanone; polyorthoester; polyanhydride; poly(glycolic acid); poly(D,L-lactic acid); poly(glycolic acid-co-trimethylene carbonate); polyphosphoester; polyphosphoester urethane; poly(amino acids); cyanoacrylates; poly(trimethylene carbonate); poly(iminocarbonate); copoly(ether-esters) (e.g.,

poly(ethylene oxide)-poly(lactic acid) (PEO/PLA) co-polymers); polyalkylene oxalates; polyphosphazenes; biomolecules, such as fibrin, fibrinogen, cellulose, starch, collagen and hyaluronic acid; polyurethanes; silicones; polyesters; polyolefins; polyisobutylene and ethylene-alphaolefin copolymers; acrylic polymers and copolymers; vinyl halide polymers and copolymers, such as polyvinyl chloride; polyvinyl ethers, such as polyvinyl methyl ether; polyvinylidene halides, such as polyvinylidene fluoride and polyvinylidene chloride; polyacrylonitrile; polyvinyl ketones; polyvinyl aromatics, such as polystyrene; polyvinyl esters, such as polyvinyl acetate; copolymers of vinyl monomers with each other and olefins, such as ethylene-methyl methacrylate copolymers, acrylonitrile-styrene copolymers, ABS resins, and ethylene-vinyl acetate copolymers; polyamides, such as Nylon 66 and polycaprolactam; alkyd resins; polycarbonates; polyoxymethylenes; polyimides; polyethers; epoxy resins; polyurethanes; rayon; rayon-triacetate; cellulose; cellulose acetate; cellulose butyrate; cellulose acetate butyrate; cellophane; cellulose nitrate; cellulose propionate; cellulose ethers; amorphous Teflon; poly(ethylene glycol); and carboxymethyl cellulose.

[00110] Suitable synthetic polymers include unsubstituted and substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol), and derivatives thereof, e.g., substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol), and derivatives thereof. Suitable naturally-occurring polymers include, e.g., albumin, amylose, dextran, glycogen, and derivatives thereof.

[00111] Suitable polymers can have an average molecular weight in a range of from 500 Da to 50000 Da, e.g., from 5000 Da to 40000 Da, or from 25000 to 40000 Da. For example, in some embodiments, where a subject antibody comprises a poly(ethylene glycol) (PEG) or methoxypoly(ethyleneglycol) polymer, the PEG or methoxypoly(ethyleneglycol) polymer can have a molecular weight in a range of from about 0.5 kiloDaltons (kDa) to 1 kDa, from about 1 kDa to 5 kDa, from 5 kDa to 10 kDa, from 10 kDa to 25 kDa, from 25 kDa to 40 kDa, or from 40 kDa to 60 kDa.

[00112] As noted above, in some embodiments, a subject antibody is covalently linked to a PEG polymer. In some embodiments, a subject scFv multimer is covalently linked to a PEG polymer. See, e.g., Albrecht et al. (2006) *J. Immunol. Methods* 310:100. Methods and reagents suitable for PEGylation of a protein are well known in the art and may be found in, e.g., U.S. Pat. No. 5,849,860. PEG suitable for conjugation to a protein is generally soluble in water at room temperature, and has the general formula $R(O-CH_2-CH_2)_nO-R$, where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. Where R is a protective group, it generally has from 1 to 8 carbons.

[00113] The PEG conjugated to the subject antibody can be linear. The PEG conjugated to the subject protein may also be branched. Branched PEG derivatives such as those described in U.S. Pat. No. 5,643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Polymers, Inc. catalog "Polyethylene Glycol Derivatives 1997-1998." Star PEGs are described in the art including, e.g., in U.S. Patent No. 6,046,305.

- [00114] A subject antibody can be glycosylated, e.g., a subject antibody can comprise a covalently linked carbohydrate or polysaccharide moiety. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.
- [00115] Addition of glycosylation sites to an antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites). Similarly, removal of glycosylation sites can be accomplished by amino acid alteration within the native glycosylation sites of an antibody.
- [00116] A subject antibody will in some embodiments comprise a "radiopaque" label, e.g. a label that can be easily visualized using for example x-rays. Radiopaque materials are well known to those of skill in the art. The most common radiopaque materials include iodide, bromide or barium salts. Other radiopaque materials are also known and include, but are not limited to organic bismuth derivatives (see, e.g., U.S. Pat. No. 5,939,045), radiopaque multiurethanes (see U.S. Pat. No. 5,346,981), organobismuth composites (see, e.g., U.S. Pat. No. 5,256,334), radiopaque barium multimer complexes (see, e.g., U.S. Pat. No. 4,866,132), and the like.
- [00117] A subject antibody can be covalently linked to a second moiety (e.g., a lipid, a polypeptide other than a subject antibody, a synthetic polymer, a carbohydrate, and the like) using for example, glutaraldehyde, a homobifunctional cross-linker, or a heterobifunctional cross-linker. Glutaraldehyde cross-links polypeptides via their amino moieties. Homobifunctional cross-linkers (e.g., a homobifunctional imidoester, a homobifunctional N-hydroxysuccinimidyl (NHS) ester, or a homobifunctional sulfhydryl reactive cross-linker) contain two or more identical reactive moieties and can be used in a one step reaction procedure in which the cross-linker is added to a solution containing a mixture of the polypeptides to be linked. Homobifunctional NHS ester and imido esters cross-link amine containing polypeptides. In a mild alkaline pH, imido esters react only with primary amines to form imidoamides, and overall charge of the cross-linked polypeptides is not affected. Homobifunctional sulfhydryl reactive cross-linkers includes bismaleimidhexane (BMH), 1,5-difluoro-2,4-dinitrobenzene (DFDNB), and 1,4-di-(3',2'-pyridyldithio) propinoamido butane (DPDPB).
- [00118] Heterobifunctional cross-linkers have two or more different reactive moieties (e.g., amine reactive moiety and a sulfhydryl-reactive moiety) and are cross-linked with one of the polypeptides via the amine or sulfhydryl reactive moiety, then reacted with the other polypeptide via the non-

reacted moiety. Multiple heterobifunctional haloacetyl cross-linkers are available, as are pyridyl disulfide cross-linkers. Carbodiimides are a classic example of heterobifunctional cross-linking reagents for coupling carboxyls to amines, which results in an amide bond.

[00119] A subject antibody can be immobilized on a solid support. Suitable supports are well known in the art and comprise, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, nylon membranes, sheets, duracytes, wells of reaction trays (e.g., multi-well plates), plastic tubes, etc. A solid support can comprise any of a variety of substances, including, e.g., glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amylose, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. Suitable methods for immobilizing a subject antibody onto a solid support are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. Solid supports can be soluble or insoluble, e.g., in aqueous solution. In some embodiments, a suitable solid support is generally insoluble in an aqueous solution.

[00120] A subject antibody will in some embodiments comprise a detectable label. Suitable detectable labels include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Suitable include, but are not limited to, magnetic beads (e.g. Dynabeads™), fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase, luciferase, and others commonly used in an enzyme-linked immunosorbent assay (ELISA)), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

[00121] In some embodiments, a subject antibody comprises a contrast agent or a radioisotope, where the contrast agent or radioisotope is one that is suitable for use in imaging, e.g., imaging procedures carried out on humans. Non-limiting examples of labels include radioisotope such as ¹²³I (iodine), ¹⁸F (fluorine), ⁹⁹Tc (technetium), ¹¹¹In (indium), and ⁶⁷Ga (gallium), and contrast agent such as gadolinium (Gd), dysprosium, and iron. Radioactive Gd isotopes (¹⁵³Gd) also are available and suitable for imaging procedures in non-human mammals. A subject antibody can be labeled using standard techniques. For example, a subject antibody can be iodinated using chloramine T or 1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycouril. For fluorination, fluorine is added to a subject antibody during the synthesis by a fluoride ion displacement reaction. See, Muller-Gartner, H., TIB Tech., 16:122-130 (1998) and Saji, H., Crit. Rev. Ther. Drug Carrier Syst., 16(2):209-244 (1999) for a review of synthesis of proteins with such radioisotopes. A subject antibody can also be labeled with a contrast agent through standard techniques. For example, a subject antibody can be labeled with Gd by conjugating low molecular Gd chelates such as Gd diethylene triamine pentaacetic acid (GdDTPA) or Gd tetraazacyclododecanetetraacetic (GdDOTA) to the antibody. See, Caravan et al., Chem. Rev.

99:2293-2352 (1999) and Lauffer et al., *J. Magn. Reson. Imaging*, 3:11-16 (1985). A subject antibody can be labeled with Gd by, for example, conjugating polylysine-Gd chelates to the antibody. See, for example, Curtet et al., *Invest. Radiol.*, 33(10):752-761 (1998). Alternatively, a subject antibody can be labeled with Gd by incubating paramagnetic polymerized liposomes that include Gd chelator lipid with avidin and biotinylated antibody. See, for example, Sipkins et al., *Nature Med.*, 4:623-626 (1998).

[00122] Suitable fluorescent proteins that can be linked to a subject antibody include, but are not limited to, a green fluorescent protein (GFP) from *Aequoria victoria* or a mutant or derivative thereof e.g., as described in U.S. Patent No. 6,066,476; 6,020,192; 5,985,577; 5,976,796; 5,968,750; 5,968,738; 5,958,713; 5,919,445; 5,874,304; e.g., Enhanced GFP, many such GFP which are available commercially, e.g., from Clontech, Inc.; a red fluorescent protein; a yellow fluorescent protein; any of a variety of fluorescent and colored proteins from Anthozoan species, as described in, e.g., Matz et al. (1999) *Nature Biotechnol.* 17:969-973; and the like.

[00123] A subject antibody will in some embodiments be linked to (e.g., covalently or non-covalently linked) a fusion partner, e.g., a ligand; an epitope tag; a peptide; a protein other than an antibody; and the like. Suitable fusion partners include peptides and polypeptides that confer enhanced stability *in vivo* (e.g., enhanced serum half-life); provide ease of purification, e.g., (His)_n, e.g., 6His, and the like; provide for secretion of the fusion protein from a cell; provide an epitope tag, e.g., glutathione-S-transferase (GST), hemagglutinin (HA; e.g., YPYDVPDYA; SEQ ID NO:34), FLAG (e.g., DYKDDDDK; SEQ ID NO:35), c-myc (e.g., EQKLISEEDL; SEQ ID NO:36), and the like; provide a detectable signal, e.g., an enzyme that generates a detectable product (e.g., β -galactosidase, luciferase), or a protein that is itself detectable, e.g., a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, etc.; provides for multimerization, e.g., a multimerization domain such as an Fc portion of an immunoglobulin; and the like.

[00124] The fusion may also include an affinity domain, including peptide sequences that can interact with a binding partner, e.g., such as one immobilized on a solid support, useful for identification or purification. Consecutive single amino acids, such as histidine, when fused to a protein, can be used for one-step purification of the fusion protein by high affinity binding to a resin column, such as nickel sepharose. Exemplary affinity domains include His5 (HHHHH) (SEQ ID NO:37), HisX6 (HHHHHH) (SEQ ID NO:38), c-myc (EQKLISEEDL) (SEQ ID NO:36), Flag (DYKDDDDK) (SEQ ID NO:35), StrepTag (WSHPQFEK) (SEQ ID NO:39), hemagglutinin, e.g., HA Tag (YPYDVPDYA; SEQ ID NO:34), glutathione-S-transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:40), Phe-His-His-Thr (SEQ ID NO:41), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:42), metal binding domains, e.g., zinc binding domains or calcium binding domains such as those from calcium-binding proteins, e.g., calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins,

parvalbumin, calbindin D9K, calbindin D28K, and calretinin, inteins, biotin, streptavidin, MyoD, leucine zipper sequences, and maltose binding protein.

[00125] A subject antibody will in some embodiments be fused to a polypeptide that binds to an endogenous blood brain barrier (BBB) receptor. Linking a subject antibody to a polypeptide that binds to an endogenous BBB receptor facilitates crossing the BBB, e.g., in a subject treatment method (see below) involving administration of a subject antibody to an individual in need thereof. Suitable polypeptides that bind to an endogenous BBB include antibodies, e.g., monoclonal antibodies, or antigen-binding fragments thereof, that specifically bind to an endogenous BBB receptor. Suitable endogenous BBB receptors include, but are not limited to, an insulin receptor, a transferrin receptor, a leptin receptor, a lipoprotein receptor, and an insulin-like growth factor receptor. See, e.g., U.S. Patent Publication No. 2009/0156498.

[00126] In some embodiments, a subject antibody comprises a polyamine modification. Polyamine modification of a subject antibody enhances permeability of the modified antibody at the BBB. A subject antibody can be modified with polyamines that are either naturally occurring or synthetic. See, for example, U.S. Pat. No. 5,670,477. Useful naturally occurring polyamines include putrescine, spermidine, spermine, 1,3-deaminopropane, norspermidine, syn-homospermidine, thermine, thermospermine, caldopentamine, homocaldopentamine, and canavalline. Putrescine, spermidine and spermine are particularly useful. Synthetic polyamines are composed of the empirical formula $C_xH_yN_z$, can be cyclic or acyclic, branched or unbranched, hydrocarbon chains of 3-12 carbon atoms that further include 1-6 NR or N(R)₂ moieties, wherein R is H, (C₁-C₄) alkyl, phenyl, or benzyl. Polyamines can be linked to an antibody using any standard crosslinking method.

[00127] In some embodiments, a subject antibody is modified to include a carbohydrate moiety, where the carbohydrate moiety can be covalently linked to the antibody. In some embodiments, a subject antibody is modified to include a lipid moiety, where the lipid moiety can be covalently linked to the antibody. Suitable lipid moieties include, e.g., an N-fatty acyl group such as N-lauroyl, N-oleoyl, etc.; a fatty amine such as dodecyl amine, oleoyl amine, etc.; a C3-C16 long-chain aliphatic lipid; and the like. See, e.g., U.S. Pat. No. 6,638,513). In some embodiments, a subject antibody is incorporated into a liposome.

Methods of producing a subject antibody

[00128] A subject antibody can be produced by any known method, e.g., conventional synthetic methods for protein synthesis; recombinant DNA methods; etc.

[00129] Where a subject antibody is a single chain polypeptide, it can be synthesized using standard chemical peptide synthesis techniques. Where a polypeptide is chemically synthesized, the synthesis may proceed via liquid-phase or solid-phase. Solid phase polypeptide synthesis (SPPS), in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence, is an example of a suitable method for the chemical synthesis of a subject antibody. Various forms of SPPS, such as Fmoc and Boc, are available

for synthesizing a subject antibody. Techniques for solid phase synthesis are described by Barany and Merrifield, *Solid-Phase Peptide Synthesis*; pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology*. Vol. 2: *Special Methods in Peptide Synthesis, Part A.*, Merrifield, et al. *J. Am. Chem. Soc.*, 85: 2149-2156 (1963); Stewart et al., *Solid Phase Peptide Synthesis*, 2nd ed. Pierce Chem. Co., Rockford, Ill. (1984); and Ganesan A. 2006 *Mini Rev. Med Chem.* 6:3-10 and Camarero JA et al. 2005 *Protein Pept Lett.* 12:723-8. Briefly, small insoluble, porous beads are treated with functional units on which peptide chains are built. After repeated cycling of coupling/deprotection, the free N-terminal amine of a solid-phase attached is coupled to a single N-protected amino acid unit. This unit is then deprotected, revealing a new N-terminal amine to which a further amino acid may be attached. The peptide remains immobilized on the solid-phase and undergoes a filtration process before being cleaved off.

[00130] Standard recombinant methods can be used for production of a subject antibody. For example, nucleic acids encoding light and heavy chain variable regions, optionally linked to constant regions, are inserted into expression vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Expression control sequences include, but are not limited to, promoters (e.g., naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences. The expression control sequences can be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells (e.g., COS or CHO cells). Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the antibodies.

[00131] Because of the degeneracy of the code, a variety of nucleic acid sequences can encode each immunoglobulin amino acid sequence. The desired nucleic acid sequences can be produced by de novo solid-phase DNA synthesis or by polymerase chain reaction (PCR) mutagenesis of an earlier prepared variant of the desired polynucleotide. Oligonucleotide-mediated mutagenesis is an example of a suitable method for preparing substitution, deletion and insertion variants of target polypeptide DNA. See Adelman et al., *DNA* 2:183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that incorporates the oligonucleotide primer, and encodes the selected alteration in the target polypeptide DNA.

[00132] Suitable expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers (e.g., ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin

resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences.

[00133] *Escherichia coli* is an example of a prokaryotic host cell that can be used for cloning a subject antibody-encoding polynucleotide. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

[00134] Other microbes, such as yeast, are also useful for expression. *Saccharomyces* (e.g., *S. cerevisiae*) and *Pichia* are examples of suitable yeast host cells, with suitable vectors having expression control sequences (e.g., promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

[00135] In addition to microorganisms, mammalian cells (e.g., mammalian cells grown in *in vitro* cell culture) can also be used to express and produce the polypeptides of the present invention (e.g., polynucleotides encoding immunoglobulins or fragments thereof). See Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y. (1987). Suitable mammalian host cells include CHO cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, and transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (Queen et al., *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Examples of suitable expression control sequences are promoters derived from immunoglobulin genes, SV40, adenovirus, bovine papilloma virus, cytomegalovirus and the like. See Co et al., *J. Immunol.* 148:1149 (1992).

[00136] Once synthesized (either chemically or recombinantly), the whole antibodies, their dimers, individual light and heavy chains, or other forms of a subject antibody (e.g., scFv, etc.) can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, high performance liquid chromatography (HPLC) purification, gel electrophoresis, and the like (see generally Scopes, *Protein Purification* (Springer-Verlag, N.Y., (1982)). A subject antibody can be substantially pure, e.g., at least about 80% to 85% pure, at least about 85% to 90% pure, at least about 90% to 95% pure, or 98% to 99%, or more, pure, e.g., free from contaminants such as cell debris, macromolecules other than a subject antibody, etc.

Compositions

[00137] The present disclosure provides a composition comprising a subject antibody. A subject antibody composition can comprise, in addition to a subject antibody, one or more of: a salt, e.g., NaCl, MgCl₂, KCl, MgSO₄, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol; and the like.

NUCLEIC ACIDS

[00138] The present disclosure provides nucleic acids comprising nucleotide sequences encoding a subject antibody. A nucleotide sequence encoding a subject antibody can be operably linked to one or more regulatory elements, such as a promoter and enhancer, that allow expression of the nucleotide sequence in the intended target cells (e.g., a cell that is genetically modified to synthesize the encoded antibody).

[00139] Suitable promoter and enhancer elements are known in the art. For expression in a bacterial cell, suitable promoters include, but are not limited to, lacI, lacZ, T3, T7, gpt, lambda P and trc. For expression in a eukaryotic cell, suitable promoters include, but are not limited to, light and/or heavy chain immunoglobulin gene promoter and enhancer elements; cytomegalovirus immediate early promoter; herpes simplex virus thymidine kinase promoter; early and late SV40 promoters; promoter present in long terminal repeats from a retrovirus; mouse metallothionein-I promoter; and various art-known tissue specific promoters.

[00140] In some embodiments, e.g., for expression in a yeast cell, a suitable promoter is a constitutive promoter such as an ADH1 promoter, a PGK1 promoter, an ENO promoter, a PYK1 promoter and the like; or a regulatable promoter such as a GAL1 promoter, a GAL10 promoter, an ADH2 promoter, a PHO5 promoter, a CUP1 promoter, a GAL7 promoter, a MET25 promoter, a MET3 promoter, a CYC1 promoter, a HIS3 promoter, an ADH1 promoter, a PGK promoter, a GAPDH promoter, an ADC1 promoter, a TRP1 promoter, a URA3 promoter, a LEU2 promoter, an ENO promoter, a TP1 promoter, and AOX1 (e.g., for use in *Pichia*). Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

[00141] Suitable promoters for use in prokaryotic host cells include, but are not limited to, a bacteriophage T7 RNA polymerase promoter; a trp promoter; a lac operon promoter; a hybrid promoter, e.g., a lac/tac hybrid promoter, a tac/trc hybrid promoter, a trp/lac promoter, a T7/lac promoter; a trc promoter; a tac promoter, and the like; an araBAD promoter; *in vivo* regulated promoters, such as an *ssaG* promoter or a related promoter (*see*, e.g., U.S. Patent Publication No. 20040131637), a *pagC* promoter (Pulkkinen and Miller, *J. Bacteriol.*, 1991: 173(1): 86-93; Alpuche-Aranda et al., PNAS, 1992; 89(21): 10079-83), a *nirB* promoter (Harborne et al. (1992) *Mol. Micro.*

6:2805-2813), and the like (*see, e.g.,* Dunstan et al. (1999) *Infect. Immun.* 67:5133-5141; McKelvie et al. (2004) *Vaccine* 22:3243-3255; and Chatfield et al. (1992) *Biotechnol.* 10:888-892); a sigma70 promoter, *e.g.,* a consensus sigma70 promoter (*see, e.g.,* GenBank Accession Nos. AX798980, AX798961, and AX798183); a stationary phase promoter, *e.g.,* a *dps* promoter, an *spv* promoter, and the like; a promoter derived from the pathogenicity island SPI-2 (*see, e.g.,* WO96/17951); an actA promoter (*see, e.g.,* Shetron-Rama et al. (2002) *Infect. Immun.* 70:1087-1096); an rpsM promoter (*see, e.g.,* Valdivia and Falkow (1996). *Mol. Microbiol.* 22:367); a tet promoter (*see, e.g.,* Hillen, W. and Wissmann, A. (1989) In Saenger, W. and Heinemann, U. (eds), *Topics in Molecular and Structural Biology, Protein-Nucleic Acid Interaction*. Macmillan, London, UK, Vol. 10, pp. 143-162); an SP6 promoter (*see, e.g.,* Melton et al. (1984) *Nucl. Acids Res.* 12:7035); and the like. Suitable strong promoters for use in prokaryotes such as *Escherichia coli* include, but are not limited to Trc, Tac, T5, T7, and P_{Lambda}. Non-limiting examples of operators for use in bacterial host cells include a lactose promoter operator (LacI repressor protein changes conformation when contacted with lactose, thereby preventing the LacI repressor protein from binding to the operator), a tryptophan promoter operator (when complexed with tryptophan, TrpR repressor protein has a conformation that binds the operator; in the absence of tryptophan, the TrpR repressor protein has a conformation that does not bind to the operator), and a tac promoter operator (*see, for example,* deBoer et al. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25).

[00142] A nucleotide sequence encoding a subject antibody can be present in an expression vector and/or a cloning vector. Where a subject antibody comprises two separate polypeptides, nucleotide sequences encoding the two polypeptides can be cloned in the same or separate vectors. An expression vector can include a selectable marker, an origin of replication, and other features that provide for replication and/or maintenance of the vector.

[00143] Large numbers of suitable vectors and promoters are known to those of skill in the art; many are commercially available for generating a subject recombinant constructs. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia).

[00144] Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins. A selectable marker operative in the expression host may be present. Suitable expression vectors include, but are not limited to, viral vectors (*e.g.* viral vectors based on vaccinia virus; poliovirus; adenovirus (*see, e.g.,* Li et al., *Invest Ophthalmol Vis Sci* 35:2543-2549, 1994; Borrás et al., *Gene Ther* 6:515-524, 1999; Li and Davidson, *PNAS* 92:7700-7704, 1995; Sakamoto et al., *Hum Gene Ther* 5:1088-1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (*see, e.g.,* Ali et al., *Hum Gene Ther* 9:81-86, 1998, Flannery et al.,

PNAS 94:6916 6921, 1997; Bennett et al., Invest Ophthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997; Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

[00145] As noted above, a subject nucleic acid comprises a nucleotide sequence encoding a subject antibody. A subject nucleic acid can comprise a nucleotide sequence encoding heavy- and light-chain anti-Sulf-2 CDRs. In some embodiments, a subject nucleic acid comprises a nucleotide sequence encoding heavy- and light-chain 8G1, 5D5, or 2E8 CDRs, where the CDR-encoding sequences are interspersed with FR-encoding nucleotide sequences. In some embodiments, the FR-encoding nucleotide sequences are human FR-encoding nucleotide sequences.

CELLS

[00146] The present disclosure provides isolated genetically modified host cells (e.g., *in vitro* cells) that are genetically modified with a subject nucleic acid. In some embodiments, a subject isolated genetically modified host cell can produce a subject antibody.

[00147] Suitable host cells include eukaryotic host cells, such as a mammalian cell, an insect host cell, a yeast cell; and prokaryotic cells, such as a bacterial cell. Introduction of a subject nucleic acid into the host cell can be effected, for example by calcium phosphate precipitation, DEAE dextran mediated transfection, liposome-mediated transfection, electroporation, or other known method.

[00148] Suitable mammalian cells include primary cells and immortalized cell lines. Suitable mammalian cell lines include human cell lines, non-human primate cell lines, rodent (e.g., mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCL1.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

[00149] Suitable yeast cells include, but are not limited to, *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*,

Trichoderma reesei, *Chrysosporium lucknowense*, *Fusarium* sp., *Fusarium gramineum*, *Fusarium venenatum*, *Neurospora crassa*, *Chlamydomonas reinhardtii*, and the like.

[00150] Suitable prokaryotic cells include, but are not limited to, any of a variety of laboratory strains of *Escherichia coli*, *Lactobacillus* sp., *Salmonella* sp., *Shigella* sp., and the like. See, e.g., Carrier et al. (1992) *J. Immunol.* 148:1176-1181; U.S. Patent No. 6,447,784; and Sizemore et al. (1995) *Science* 270:299-302. Examples of *Salmonella* strains which can be employed in the present invention include, but are not limited to, *Salmonella typhi* and *S. typhimurium*. Suitable *Shigella* strains include, but are not limited to, *Shigella flexneri*, *Shigella sonnei*, and *Shigella dysenteriae*. Typically, the laboratory strain is one that is non-pathogenic. Non-limiting examples of other suitable bacteria include, but are not limited to, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas mevalonii*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Rhodococcus* sp., and the like. In some embodiments, the host cell is *Escherichia coli*.

COMPOSITIONS

[00151] The present disclosure provides compositions, including pharmaceutical compositions, comprising a subject antibody. In general, a formulation comprises an effective amount of a subject antibody. In the context of cancer treatment, an "effective amount" means a dosage sufficient to produce a desired result, e.g., reduction in tumor growth, reduction in number of cancer cells, reduction in tumor mass, etc. Generally, the desired result is at least a reduction in one or more of tumor growth, cancer cell number, and tumor mass, as compared to a control. A subject antibody can be delivered in such a manner as to avoid the blood-brain barrier, as described in more detail below. A subject antibody can be formulated and/or modified to enable the antibody to cross the blood-brain barrier.

Formulations

[00152] In the subject methods, a subject antibody can be administered to the host using any convenient means capable of resulting in the desired therapeutic effect or diagnostic effect. Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, a subject antibody can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

[00153] In pharmaceutical dosage forms, a subject antibody can be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

[00154] For oral preparations, a subject antibody can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose,

cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

[00155] A subject antibody can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[00156] Pharmaceutical compositions comprising a subject antibody are prepared by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, stabilizers, surfactants, buffers and/or tonicity agents. Acceptable carriers, excipients and/or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid, glutathione, cysteine, methionine and citric acid; preservatives (such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, or combinations thereof); amino acids such as arginine, glycine, ornithine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophan, methionine, serine, proline and combinations thereof; monosaccharides, disaccharides and other carbohydrates; low molecular weight (less than about 10 residues) polypeptides; proteins, such as gelatin or serum albumin; chelating agents such as EDTA; sugars such as trehalose, sucrose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, N-methylglucosamine, galactosamine, and neuraminic acid; and/or non-ionic surfactants such as Tween, Brij Pluronics, Triton-X, or polyethylene glycol (PEG).

[00157] The pharmaceutical composition may be in a liquid form, a lyophilized form or a liquid form reconstituted from a lyophilized form, wherein the lyophilized preparation is to be reconstituted with a sterile solution prior to administration. The standard procedure for reconstituting a lyophilized composition is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization); however solutions comprising antibacterial agents may be used for the production of pharmaceutical compositions for parenteral administration; see also Chen (1992) Drug Dev Ind Pharm 18, 1311-54.

[00158] Exemplary antibody concentrations in a subject pharmaceutical composition may range from about 1 mg/mL to about 200 mg/ml or from about 50 mg/mL to about 200 mg/mL, or from about 150 mg/mL to about 200 mg/mL.

[00159] An aqueous formulation of the antibody may be prepared in a pH-buffered solution, e.g., at pH ranging from about 4.0 to about 7.0, or from about 5.0 to about 6.0, or alternatively about 5.5. Examples of buffers that are suitable for a pH within this range include phosphate-, histidine-, citrate-, succinate-, acetate-buffers and other organic acid buffers. The buffer concentration can be from about

1 mM to about 100 mM, or from about 5 mM to about 50 mM, depending, e.g., on the buffer and the desired tonicity of the formulation.

[00160] A tonicity agent may be included in the antibody formulation to modulate the tonicity of the formulation. Exemplary tonicity agents include sodium chloride, potassium chloride, glycerin and any component from the group of amino acids, sugars as well as combinations thereof. In some embodiments, the aqueous formulation is isotonic, although hypertonic or hypotonic solutions may be suitable. The term "isotonic" denotes a solution having the same tonicity as some other solution with which it is compared, such as physiological salt solution or serum. Tonicity agents may be used in an amount of about 5 mM to about 350 mM, e.g., in an amount of 100 mM to 350 mM.

[00161] A surfactant may also be added to the antibody formulation to reduce aggregation of the formulated antibody and/or minimize the formation of particulates in the formulation and/or reduce adsorption. Exemplary surfactants include polyoxyethylenesorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers (Brij), alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene copolymer (Poloxamer, Pluronic), and sodium dodecyl sulfate (SDS). Examples of suitable polyoxyethylenesorbitan-fatty acid esters are polysorbate 20, (sold under the trademark Tween 20™) and polysorbate 80 (sold under the trademark Tween 80™). Examples of suitable polyethylene-polypropylene copolymers are those sold under the names Pluronic® F68 or Poloxamer 188™. Examples of suitable Polyoxyethylene alkyl ethers are those sold under the trademark Brij™. Exemplary concentrations of surfactant may range from about 0.001% to about 1% w/v.

[00162] A lyoprotectant may also be added in order to protect the labile active ingredient (e.g. a protein) against destabilizing conditions during the lyophilization process. For example, known lyoprotectants include sugars (including glucose and sucrose); polyols (including mannitol, sorbitol and glycerol); and amino acids (including alanine, glycine and glutamic acid). Lyoprotectants can be included in an amount of about 10 mM to 500 mM.

[00163] In some embodiments, a subject formulation includes a subject antibody, and one or more of the above-identified agents (e.g., a surfactant, a buffer, a stabilizer, a tonicity agent) and is essentially free of one or more preservatives, such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, and combinations thereof. In other embodiments, a preservative is included in the formulation, e.g., at concentrations ranging from about 0.001 to about 2% (w/v).

[00164] For example, a subject formulation can be a liquid or lyophilized formulation suitable for parenteral administration, and can comprise: about 1 mg/mL to about 200 mg/mL of a subject antibody; about 0.001 % to about 1 % of at least one surfactant; about 1 mM to about 100 mM of a buffer; optionally about 10 mM to about 500 mM of a stabilizer; and about 5 mM to about 305 mM of a tonicity agent; and has a pH of about 4.0 to about 7.0.

- [00165] As another example, a subject parenteral formulation is a liquid or lyophilized formulation comprising: about 1 mg/mL to about 200 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM Sucrose; and has a pH of 5.5.
- [00166] As another example, a subject parenteral formulation comprises a lyophilized formulation comprising: 1) 15 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM sucrose; and has a pH of 5.5; or 2) 75 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM sucrose; and has a pH of 5.5; or 3) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM Sucrose; and has a pH of 5.5; or 4) 75 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 6) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5.
- [00167] As another example, a subject parenteral formulation is a liquid formulation comprising: 1) 7.5 mg/mL of a subject antibody; 0.022% Tween 20 w/v; 120 mM L-histidine; and 250 mM sucrose; and has a pH of 5.5; or 2) 37.5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 10 mM L-histidine; and 125 mM sucrose; and has a pH of 5.5; or 3) 37.5 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 10 mM L-histidine; and 125 mM sucrose; and has a pH of 5.5; or 4) 37.5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 10 mM L-histidine; 125 mM trehalose; and has a pH of 5.5; or 5) 37.5 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 10 mM L-histidine; and 125 mM trehalose; and has a pH of 5.5; or 6) 5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 7) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM mannitol; and has a pH of 5.5; or 8) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 140 mM sodium chloride; and has a pH of 5.5; or 9) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 10) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM mannitol; and has a pH of 5.5; or 11) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 140 mM sodium chloride; and has a pH of 5.5; or 12) 10 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 20 mM L-histidine; and 40 mM sodium chloride; and has a pH of 5.5.
- [00168] A subject antibody can be utilized in aerosol formulation to be administered via inhalation. A subject antibody can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.
- [00169] Furthermore, a subject antibody can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. A subject antibody can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.
- [00170] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or

suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may comprise a subject antibody in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

[00171] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for a subject antibody may depend on the particular antibody employed and the effect to be achieved, and the pharmacodynamics associated with each antibody in the host.

[00172] Other modes of administration will also find use with the subject invention. For instance, a subject antibody can be formulated in suppositories and, in some cases, aerosol and intranasal compositions. For suppositories, the vehicle composition will include traditional binders and carriers such as, polyalkylene glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), e.g., about 1% to about 2%.

[00173] Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

[00174] A subject antibody can be administered as an injectable formulation. Typically, injectable compositions are prepared as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the antibody encapsulated in liposome vehicles.

[00175] Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of a subject antibody adequate to achieve the desired state in the subject being treated.

[00176] The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

[00177] In some embodiments, a subject antibody is formulated in a controlled release formulation. Sustained-release preparations may be prepared using methods well known in the art. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody in which the matrices are in the form of shaped articles, e.g. films or microcapsules. Examples of sustained-release matrices include polyesters, copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, hydrogels, polylactides, degradable lactic acid-glycolic acid copolymers and poly-D-(-)-3-hydroxybutyric acid. Possible loss of biological activity and possible changes in immunogenicity of antibodies comprised in sustained-release preparations may be prevented by using appropriate additives, by controlling moisture content and by developing specific polymer matrix compositions.

[00178] Controlled release within the scope of this invention can be taken to mean any one of a number of extended release dosage forms. The following terms may be considered to be substantially equivalent to controlled release, for the purposes of the present invention: continuous release, controlled release, delayed release, depot, gradual release, long-term release, programmed release, prolonged release, proportionate release, protracted release, repository, retard, slow release, spaced release, sustained release, time coat, timed release, delayed action, extended action, layered-time action, long acting, prolonged action, repeated action, slowing acting, sustained action, sustained-action medications, and extended release. Further discussions of these terms may be found in Lesczek Krowczynski, Extended-Release Dosage Forms, 1987 (CRC Press, Inc.).

[00179] The various controlled release technologies cover a very broad spectrum of drug dosage forms. Controlled release technologies include, but are not limited to physical systems and chemical systems.

[00180] Physical systems include, but are not limited to, reservoir systems with rate-controlling membranes, such as microencapsulation, macroencapsulation, and membrane systems; reservoir systems without rate-controlling membranes, such as hollow fibers, ultra microporous cellulose triacetate, and porous polymeric substrates and foams; monolithic systems, including those systems physically dissolved in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable), and materials physically dispersed in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable); laminated structures, including reservoir layers chemically similar or dissimilar to outer control layers; and other physical methods, such as osmotic pumps, or adsorption onto ion-exchange resins.

[00181] Chemical systems include, but are not limited to, chemical erosion of polymer matrices (e.g., heterogeneous, or homogeneous erosion), or biological erosion of a polymer matrix (e.g., heterogeneous, or homogeneous). Additional discussion of categories of systems for controlled release may be found in Agis F. Kydonieus, Controlled Release Technologies: Methods, Theory and Applications, 1980 (CRC Press, Inc.).

[00182] There are a number of controlled release drug formulations that are developed for oral administration. These include, but are not limited to, osmotic pressure-controlled gastrointestinal delivery systems; hydrodynamic pressure-controlled gastrointestinal delivery systems; membrane permeation-controlled gastrointestinal delivery systems, which include microporous membrane permeation-controlled gastrointestinal delivery devices; gastric fluid-resistant intestine targeted controlled-release gastrointestinal delivery devices; gel diffusion-controlled gastrointestinal delivery systems; and ion-exchange-controlled gastrointestinal delivery systems, which include cationic and anionic drugs. Additional information regarding controlled release drug delivery systems may be found in Yie W. Chien, Novel Drug Delivery Systems, 1992 (Marcel Dekker, Inc.). Some of these formulations will now be discussed in more detail..

Dosages

[00183] A suitable dosage can be determined by an attending physician or other qualified medical personnel, based on various clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex of the patient, time, and route of administration, general health, and other drugs being administered concurrently. A subject antibody may be administered in amounts between 1 ng/kg body weight and 20 mg/kg body weight per dose, e.g. between 0.1 mg/kg body weight to 10 mg/kg body weight, e.g. between 0.5 mg/kg body weight to 5 mg/kg body weight; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it can also be in the range of 1 µg to 10 mg per kilogram of body weight per minute.

[00184] Those of skill will readily appreciate that dose levels can vary as a function of the specific antibody, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Routes of administration

[00185] A subject antibody is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[00186] Conventional and pharmaceutically acceptable routes of administration include intratumoral, peritumoral, intramuscular, intratracheal, intracranial, subcutaneous, intradermal, topical application, intravenous, intraarterial, rectal, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the antibody and/or the desired effect. A subject antibody composition can be administered in a single dose or in multiple doses. In some embodiments, a subject antibody composition is administered orally. In some embodiments, a subject antibody composition is administered via an inhalational route. In some embodiments, a subject antibody composition is administered intranasally. In some embodiments, a

subject antibody composition is administered locally. In some embodiments, a subject antibody composition is administered intratumorally. In some embodiments, a subject antibody composition is administered peritumorally. In some embodiments, a subject antibody composition is administered intracranially. In some embodiments, a subject antibody composition is administered intravenously.

[00187] The agent can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

[00188] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of a subject antibody. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

[00189] A subject antibody can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

[00190] By treatment is meant at least an amelioration of the symptoms associated with the pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, *e.g.* symptom, associated with the pathological condition being treated, such as cancer, and pain associated therewith, or type 2 diabetes. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, *e.g.* prevented from happening, or stopped, *e.g.* terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

[00191] In some embodiments, a subject antibody is administered by injection and/or delivery, *e.g.*, to a site in a brain artery or directly into brain tissue. A subject antibody can also be administered directly to a target site *e.g.*, by biolistic delivery to the target site.

[00192] A variety of hosts (wherein the term “host” is used interchangeably herein with the terms “subject,” “individual,” and “patient”) are treatable according to the subject methods. Generally such hosts are “mammals” or “mammalian,” where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (*e.g.*, dogs and cats), rodentia (*e.g.*, mice, guinea pigs, and rats), and primates (*e.g.*, humans, chimpanzees, and monkeys). In some embodiments, the hosts will be humans.

[00193] Kits with unit doses of a subject antibody, *e.g.* in oral or injectable doses, are provided. In such kits, in addition to the containers containing the unit doses will be an informational package

insert describing the use and attendant benefits of the antibody in treating pathological condition of interest. Preferred compounds and unit doses are those described herein above.

TREATMENT METHODS

[00194] The present disclosure provides treatment methods, involving administering to an individual in need thereof an effective amount of a subject anti-Sulf-2 antibody.

Cancer treatment

[00195] The present disclosure provides methods of reducing proliferation of a cancer cell in an individual, where the cancer cell over-expresses a Sulf-2 polypeptide, e.g., where the cancer cell is a pancreatic cancer cell, a breast cancer cell, a non-small-cell lung cancer cell (lung adenocarcinoma and lung squamous cell carcinoma), a hepatocellular carcinoma, a central nervous system neoplasm, a gastric carcinoma, a skin cancer cell, a head & neck carcinoma, a kidney cancer cell, or a multiple myeloma. The methods generally involve contacting the cell with a subject anti-Sulf-2 antibody, e.g., administering to an individual having a cancer an effective amount of a subject anti-Sulf-2 antibody.

[00196] In some embodiments, an effective amount of a subject anti-Sulf-2 antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) is effective to reduce proliferation of the cancer cell by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more than 80%, compared to the level of proliferation of the cancer cell in absence of treatment with a subject anti-Sulf-2 antibody.

[00197] The present disclosure provides methods of treating cancer in an individual, the methods generally involving administering to an individual in need thereof (e.g., an individual having a cancer; e.g., an individual having a cancer characterized by cancer cells that over-express Sulf-2) an effective amount of a subject antibody, alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents.

[00198] In some embodiments, an effective amount of a subject antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce one or more of tumor growth rate, cancer cell number, and tumor mass, by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the tumor growth rate, cancer cell number, or tumor mass in the absence of treatment with the antibody.

[00199] Cancers that can be treated using a subject anti-Sulf-2 antibody include, but are not limited to, pancreatic cancer, breast cancer, non-small-cell lung cancer (lung adenocarcinoma and lung squamous cell carcinoma), hepatocellular carcinoma, pancreatic cancer, central nervous system neoplasms, breast cancer, gastric carcinoma, skin cancer, head & neck carcinoma, kidney cancer, and multiple myeloma.

- [00200]** In some cases, cancers suitable for treatment with a subject anti-Sulf-2 antibody include cancers that over-express Sulf-2 based on staining of biopsies, in situ imaging of Sulf-2 protein Sulf-2 enzymatic activity in nascent tumors, or increased levels of shed Sulf-2 protein in bodily fluids (e.g., blood, plasma, bronchoalveolar lavage fluid, pancreatic ductal fluid, nipple aspirate fluid, etc.).
- [00201]** In some embodiments, a subject anti-Sulf-2 antibody is administered as an adjuvant therapy to a standard cancer therapy. Standard cancer therapies include surgery (e.g., surgical removal of cancerous tissue), radiation therapy, bone marrow transplantation, chemotherapeutic treatment, biological response modifier treatment, and certain combinations of the foregoing.
- [00202]** Radiation therapy includes, but is not limited to, x-rays or gamma rays that are delivered from either an externally applied source such as a beam, or by implantation of small radioactive sources.
- [00203]** Chemotherapeutic agents are non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones.
- [00204]** Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (Cytoxan™), melphalan (L-sarcosine), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.
- [00205]** Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatin, and gemcitabine.
- [00206]** Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol®), docetaxel (Taxotere®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, *e.g.* vincristine, vinblastine, vinorelbine, vindesine, *etc.*; podophyllotoxins, *e.g.* etoposide, teniposide, *etc.*; antibiotics, *e.g.* anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, *etc.*; phenoxizone biscyclopeptides, *e.g.* dactinomycin; basic glycopeptides, *e.g.* bleomycin; anthraquinone glycosides, *e.g.* plicamycin (mithramycin); anthracenediones, *e.g.* mitoxantrone; azirinopyrrolo indolediones, *e.g.* mitomycin; macrocyclic immunosuppressants, *e.g.* cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, *etc.*; and the like.

- [00207] Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrozole, letrozole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.
- [00208] Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol®), Taxol® derivatives, docetaxel (Taxotere®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, eopthilone B, discodermolide; estramustine, nocodazole, and the like.
- [00209] Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, *e.g.* prednisone, dexamethasone, *etc.*; estrogens and pregestins, *e.g.* hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; *etc.*; and adrenocortical suppressants, *e.g.* aminoglutethimide; 17 α -ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex®. Estrogens stimulate proliferation and differentiation, therefore compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.
- [00210] Other chemotherapeutic agents include metal complexes, *e.g.* cisplatin (cis-DDP), carboplatin, *etc.*; ureas, *e.g.* hydroxyurea; and hydrazines, *e.g.* N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; *etc.* Other anti-proliferative agents of interest include immunosuppressants, *e.g.* mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); *etc.*
- [00211] "Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yannanensis*).
- [00212] Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., Taxotere™ docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

[00213] Also included within the term “taxane” are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

[00214] Biological response modifiers suitable for use in connection with the methods of the invention include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) IFN- α ; (7) IFN- γ (8) colony-stimulating factors; and (9) inhibitors of angiogenesis.

Type 2 diabetes

[00215] The present disclosure provides methods of treating postprandial dyslipoproteinemia associated with type 2 diabetes, the methods generally involving administering to an individual in need thereof (e.g., an individual having type 2 diabetes) an effective amount of a subject antibody, alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents.

[00216] In some embodiments, an effective amount of a subject antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce one or more symptoms of Type 2 diabetes, e.g., reduce body weight to within a normal range, reduce postprandial dyslipoproteinemia, reduce plasma low density lipoprotein (LDL) cholesterol levels, reduce plasma triglyceride levels, etc.

[00217] In some embodiments, an effective amount of a subject anti-Sulf-2 antibody is an amount that, when administered to an individual alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce plasma low density lipoprotein (LDL) cholesterol levels in the individual by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50%, compared to the plasma LDL cholesterol level in the individual not treated with a subject antibody. In some embodiments, an effective amount of a subject anti-Sulf-2 antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce plasma LDL cholesterol levels to within a normal range, e.g., to below 100 mg/dL. In some embodiments, the individual has type 2 diabetes, or is considered at risk for developing type 2 diabetes.

[00218] In some embodiments, an effective amount of a subject anti-Sulf-2 antibody is an amount that, when administered to an individual alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce plasma triglyceride (TG) levels in the individual by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50%, compared to the plasma TG levels in the individual not treated with a subject antibody. In some embodiments, an effective amount of a subject anti-Sulf-2 antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce plasma TG levels to within a normal range, e.g., to less than 150 mg/dL. In some embodiments, the individual has type 2 diabetes, or is considered at risk for developing type 2 diabetes.

[00219] In some embodiments, an effective amount of a subject anti-Sulf-2 antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce postprandial dyslipoproteinemia.

[00220] In some embodiments, a subject treatment method involves administering a subject antibody and one or more additional therapeutic agents. Suitable additional therapeutic agents include, but are not limited to, i) PPAR γ agonists such as glitazones (for example ciglitazone, darglitazone, englitazone, isaglitazone (MCC-555), pioglitazone, rosiglitazone, troglitazone, BRL49653, CLX-0921 and 5-BTSD), GW-0207, LG-100641 and LY-300512; (ii) biguanides such as buformin, metformin and phenformin; (iii) protein tyrosine phosphatase-1B (PTP-1B) inhibitors; (iv) sulfonylureas such as acetohexamide, chlorpropamide, diabinese, glybenclamide, glipizide, glyburide, glimepiride, gliclazide, glipentide, gliquidone, glisolamide, tolazamide and tolbutamide; (v) meglitinides such as repaglinide and nateglinide; (vi) alpha glucoside hydrolase inhibitors such as acarbose, adiposine, camiglibose, emiglitate, miglitol, voglibose, pradimicin-Q, salbostatin, CKD-711, MDL-25,637, MDL-73,945 and MOR14; (vii) alpha-amylase inhibitors such as tendamistat, trestatin and Al-3688; (viii) insulin secretagogues such as linogiride and A-4166; (ix) fatty acid oxidation inhibitors, such as clomoxir and etomoxir; (x) A2 antagonists, such as midaglizole, isaglidole, deriglidole, idazoxan, earoxan and fluparoxan; (xi) insulin or insulin mimetics, such as biota, LP-100, novarapid, insulin detemir, insulin lispro, insulin glargine, insulin zinc suspension (lente and ultralente), Lys-Pro insulin, GLP-1 (73-7) (insulin tropin) and GLP-1 (7-36)-NH₂; (xii) non-thiazolidinediones such as JT-501 and farglitazar (GW-2570/GI-262579); (xiii) PPAR α/γ dual agonists such as MK-0767, CLX-0940, GW-1536, GW-1929, GW-2433, KRP-297, L-796449, LR-90 and SB219994; (xiv) other insulin sensitizing drugs; and (xv) VPAC2 receptor agonists.

SUBJECTS SUITABLE FOR TREATMENT

[00221] A variety of subjects are suitable for treatment with a subject method of treating cancer. Suitable subjects include any individual, e.g., a human, who has cancer, who has been diagnosed with

cancer, who is at risk for developing cancer, who has had cancer and is at risk for recurrence of the cancer, who has been treated with an agent other than a subject anti-Sulf-2 antibody for the cancer and failed to respond to such treatment, or who has been treated with an agent other than a subject anti-Sulf-2 antibody for the cancer but relapsed after initial response to such treatment.

[00222] Subjects who are suitable for treatment with a subject method for treating dyslipoproteinemia associated with type 2 diabetes include individuals who have been diagnosed as having type 2 diabetes; and individuals who have type 2 diabetes, who have been treated with an agent other than a subject anti-Sulf-2 antibody for the type 2 diabetes, and who either failed to respond to such treatment or initially responded and then relapsed.

DETECTION METHODS

[00223] The present disclosure provides various detection methods that involve use of a subject antibody. Detection methods include diagnostic methods, prognostic methods, and monitoring methods.

[00224] In some embodiments, a subject detection method is carried out *in vitro*. For example, an *in vitro* detection method generally involves detecting a sulfatase-2 (Sulf-2) polypeptide in a biological sample. For example, in some cases, a subject detection method comprises: a) contacting a biological sample with a subject anti-Sulf-2 antibody; and b) detecting binding, if any, of the antibody to an epitope present in the sample.

[00225] In some embodiments, the anti-Sulf-2 antibody comprises a detectable label, and detection of binding of the antibody to an epitope present in the sample involves detecting the detectable label. Any of a variety of immunological assays can be used, including, e.g., an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay, a protein blot assay, and the like. In some cases, a subject anti-Sulf-2 antibody is immobilized on a solid support, e.g., a well of a 96-well plate, a test strip, and the like. For example, a first subject anti-Sulf-2 antibody is immobilized in a region of a test strip; a liquid biological sample is applied to the test strip, allowing contact between molecules in the biological sample and the immobilized antibodies, such that, if present, a Sulf-2 polypeptide forms a complex with the immobilized anti-Sulf-2 antibody; and a second, detectably labeled anti-Sulf-2 antibody is contacted with the complex. Binding of the second, detectably labeled anti-Sulf-2 antibody to the Sulf-2 polypeptide in the complex can be detected using an assay appropriate to the detectable label. For example, where the detectable label is an enzyme, the assay can be a colorimetric or fluorimetric assay.

[00226] In carrying out a subject *in vitro* detection assay, a subject antibody can be immobilized on a solid support. Suitable supports are well known in the art and comprise, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, nylon membranes, sheets, duracytes, wells of reaction trays (e.g., multi-well plates), plastic tubes, etc. A solid support can comprise any of a variety of substances, including, e.g., glass, polystyrene, polyvinyl chloride, polypropylene,

polyethylene, polycarbonate, dextran, nylon, amylose, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. Suitable methods for immobilizing a subject antibody onto a solid support are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. Solid supports can be soluble or insoluble, e.g., in aqueous solution. In some embodiments, a suitable solid support is generally insoluble in an aqueous solution.

[00227] In carrying out a subject *in vitro* or *in vivo* detection method, a subject antibody will in some embodiments comprise a detectable label. Suitable detectable labels include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Suitable include, but are not limited to, magnetic beads (e.g. Dynabeads™), fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase, luciferase, and others commonly used in an enzyme-linked immunosorbent assay (ELISA)), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

[00228] In some embodiments, a subject detection method is carried out *in vivo*. For example, a subject anti-Sulf-2 antibody can comprise a contrast agent or radioisotope, where the contrast agent or radioisotope is one that is suitable for use in imaging, e.g., imaging procedures carried out on humans. For example, in some cases, a subject anti-Sulf-2 antibody comprises a contrast agent or radioisotope suitable for use in imaging; and the antibody is administered to a subject. Detection of binding of the anti-Sulf-2 antibody is carried out using an appropriate method, including, e.g., magnetic resonance imaging or a radiological method.

[00229] Non-limiting examples of labels include radioisotope such as ¹²³¹I (iodine), ¹⁸F (fluorine), ⁹⁹Tc (technetium), ¹¹¹In (indium), and ⁶⁷Ga (gallium), and contrast agent such as gadolinium (Gd), dysprosium, and iron. Radioactive Gd isotopes (¹⁵³Gd) also are available and suitable for imaging procedures in non-human mammals. A subject antibody can be labeled using standard techniques. For example, a subject antibody can be iodinated using chloramine T or 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril. For fluorination, fluorine is added to a subject antibody during the synthesis by a fluoride ion displacement reaction. See, Muller-Gartner, H., TIB Tech., 16:122-130 (1998) and Saji, H., Crit. Rev. Ther. Drug Carrier Syst., 16(2):209-244 (1999) for a review of synthesis of proteins with such radioisotopes. A subject antibody can also be labeled with a contrast agent through standard techniques. For example, a subject antibody can be labeled with Gd by conjugating low molecular Gd chelates such as Gd diethylene triamine pentaacetic acid (GdDTPA) or Gd tetraazacyclododecanetetraacetic (GdDOTA) to the antibody. See, Caravan et al., Chem. Rev. 99:2293-2352 (1999) and Lauffer et al., J. Magn. Reson. Imaging, 3:11-16 (1985). A subject antibody can be labeled with Gd by, for example, conjugating polylysine-Gd chelates to the antibody. See, for example, Curtet et al., Invest. Radiol., 33(10):752-761 (1998). Alternatively, a subject antibody can be labeled with Gd by incubating paramagnetic polymerized liposomes that include Gd chelator lipid

with avidin and biotinylated antibody. See, for example, Sipkins et al., Nature Med., 4:623-626 (1998).

[00230] In some embodiments, a subject method is a diagnostic method, e.g., to determine whether an individual has a cancer that overexpresses a Sulf-2 polypeptide. In some embodiments, a subject detection method can detect a cancer.

[00231] In some embodiments, a subject method is a monitoring method, e.g., an individual who has been diagnosed as having a cancer (as described above) and is being treated for the cancer, is monitored for response to the treatment and/or progression/regression of the disorder.

KITS

[00232] The present disclosure provides a kit (e.g., a test kit) that includes a subject antibody. A subject kit is useful in carrying out a subject detection method.

[00233] A subject kit includes a subject anti-Sulf-2 antibody. The subject antibody in a subject kit can be humanized. A subject kit can include reagents for labeling the antibody. In some embodiments, the antibody in a subject kit comprises a detectable label.

[00234] Other optional components of the kit include: a buffer; a protease inhibitor; a detectable label; etc. The various components of the kit may be present in separate containers or certain compatible components may be pre-combined into a single container, as desired.

[00235] In addition to above-mentioned components, a subject kit can include instructions for using the components of the kit to practice a subject method. The instructions for practicing a subject method are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. compact disc-read only memory (CD-ROM), digital versatile disk (DVD), diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

EXAMPLES

[00236] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some

experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Generation and characterization of antibodies to Sulf-2

METHODS

Production of monoclonal antibodies

- [00237] Preparation of the immunogen. HEK293 cells were transiently transfected with pcDNA3.1/Myc-His(-)-HSulf-2 (6) using FuGENE 6 (Roche). This plasmid encodes the full-length human Sulf-2 protein sequence (GenBank protein accession number NP_940998, the b isoform containing 867 amino acids). The conditioned medium was collected 72 hours later and clarified by centrifugation. Recombinant human Sulf-2 protein bound to nickel beads (Qiagen) and was eluted with 250 mM imidazole, 300 mM NaCl in 50 mM NaH₂PO₄, pH 8 with 0.05% Tween-20. This material was concentrated and washed into Dulbecco's PBS.
- [00238] Immunization Protocol. 30 µg of immunogen, in complete Freund's adjuvant (CFA), was injected subcutaneously into a Sulf-2 null mouse on the FVB/N background (18), followed 22 days later by a second subcutaneous injection of 30 µg of immunogen in incomplete Freund's adjuvant. 15 µg of immunogen was injected intraperitoneally 19 days later and the mouse sacrificed four days after the final boost. Splenocytes were fused with the F0 mouse myeloma cell line and hybridomas were generated.
- [00239] Screening Protocol. The screening strategy consisted of positive enzyme-linked immunosorbent assay (ELISA) detection of both recombinant and native human Sulf-2, as well as negative ELISA detection of control material. The recombinant material was produced by transient transfection of HEK293 cells as described above in the preparation of the immunogen. Control material was HEK293 cell conditioned medium that did not undergo transfection. Native material was obtained from MCF7 cells grown to approximately 70% confluency in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1 µg/ml insulin. The medium was removed and the cells grown for 3 to 4 days in OptiMEM I. The resulting conditioned medium is coated onto ELISA plates. After blocking with bovine serum albumin (BSA) in PBS, the hybridoma conditioned medium is applied, followed by alkaline phosphatase-conjugated goat anti-mouse IgG and para-nitro phenyl phosphate (PNPP) for colorimetric detection. Cells in selected wells were expanded and subjected to two rounds of single-cell subcloning. ELISAs with purified mAbs (Fig. 1) were performed with MCF7-conditioned medium coated onto plastic wells using the same procedures as described above.

In Vitro Assays of Malignant Phenotype

[00240] Cell growth. H292 is a non-small lung carcinoma cell derived from a human pulmonary carcinoma with features of squamous cell carcinoma. H292 cells were originally obtained from the American Type Culture Collection. The cells were grown in RPMI-1640 supplemented with 10% FBS and penicillin/streptomycin. ≈ 1000 cells were seeded per well of a 96 well plate. Cultures were maintained in a 37°C incubator in a humidified atmosphere of 5% CO₂. Medium was removed and replaced with fresh medium every 2 days. Antibodies or control IgGs were added at the indicated final concentration. Cell growth was monitored at the indicated times the by Cell Titer Blue cell viability assay (Promega) with readings taken on a microplate reader at 490 nm. Readings for each condition were performed with 6 replicates.

[00241] In some experiments, H292 cells were transduced with a short hairpin RNA (shRNA) via a lentivirus construct in order to knockdown the expression of Sulf-2 protein. The following target region: GCTGAAGCTGCATAAGTGC (SEQ ID NO:43) was used for the design of the Sulf-2 shRNA as described in detail previously (19, 23). The control shRNA targeted an irrelevant sequence (AACAGTCGCGTTTGCGACTGG; SEQ ID NO:44). The hairpin oligonucleotides were cloned into the lentiviral shRNA expression vector pLVTHM, which encodes the inserted shRNA and GFP. Lentivirus production and infection was done as described (19). H292 cells were transduced with the virus and sorted for GFP to obtain successfully transduced cells. The Sulf-2 shRNA reduced Sulf-2 protein expression in H292 cells by >90% (23). H292 cells with Sulf-2 knockdown or treated with the control shRNA were evaluated in the cell growth assay for the effects of the antibodies 8G1 and 5D5. On day 0, cells were exposed to the indicated Sulf2 mAbs (25 μ g/ml), mIgG2b (25 μ g/ml), or PBS (no treatment). Abs were replenished at the time of medium change every 2 days. After 6 days of incubation at 37°C, counts of viable cells were determined with the Cell Titer Blue assay as above. Six replicates were measured for each treatment.

[00242] Cell migration assay. Assays were carried out as described before (23). H292 cells were grown to confluency in 6-well plates. Scratches were made with a pipette tip. The wells had the indicated additions: Sulf-2 mAbs (8G1, 5D5, or 2E8 at 25 μ g/ml final concentration), or irrelevant IgGs (mIgG_{2b} or mIgG₁ at 25 μ g/ml final concentration) or control (PBS). Cell migration was quantified by measuring the width of the wounds at 0, 24, and 48 hours. 4 replicates per treatment were measured.

[00243] Colony formation in soft agar. Assays were carried out as described before (23). 1000 H292 cells were seeded into 60-mm dishes in a suspension of 0.5% bacto-agar (Difco) in medium supplemented with 10% fetal calf serum on top of a bed of 0.5% bacto-agar (Difco) in the same medium. Cells were plated on top of 1 ml of 0.5% agarose. The plates had the indicated additions: Sulf-2 mAbs (8G1, 5D5, or 2E8 at 25 μ g/ml final concentration), or irrelevant IgGs (mIgG_{2b} or mIgG₁ at 25 μ g/ml final concentration) or control (PBS). Plates were incubated at 37°C in 5% CO₂, and

colonies that had formed after 21 days of culture were counted. Triplicate determinations were performed for each condition.

In Vivo Assay of Tumorigenicity

[00244] Pharmacokinetics of 8G1 mAb. To determine the half-life of 8G1, the antibody was injected intraperitoneally into male BALB/c nu/nu mice (at 20 mg/kg). 3 mice were injected. At various times, the mice were bled to obtain serum. The level of 8G1 was determined by performing an ELISA against coated human Sulf-2. Comparison with a standard curve obtained with purified 8G1 allowed the determination of 8G1 levels in the sera. The half-life was determined to be 48 hrs.

[00245] Tumorigenicity assay. A total of 5×10^6 H292 cells were injected subcutaneously into the flanks of 5-week-old athymic, male, BALB/c nu/nu mice (five mice per group) according to the previously described procedures (23). The mice were approximately 25 gm in weight. Tumors were allowed to grow to a palpable size ($>200 \text{ mm}^3$) and then treatments were commenced. Five mice received intraperitoneal injections of the Sulf-2 mAb (8G1 at 0.5 mg per dose), five mice got control treatment (vehicle PBS alone) and five mice received control treatment (MOPC-21, an irrelevant mIgG₁ at 0.5 mg per dose), three times per week. Tumor size was monitored twice a week by calipers and tumor volumes were computed. At termination, tumors were excised and weighed. At termination, the serum level of 8G1 was determined to be $19 \pm 6 \text{ } \mu\text{g/ml}$ (mean $\pm$ SEM) for the 5 mice.

RESULTS

[00246] *Preparation of monoclonal antibodies.* Monoclonal antibodies against Sulf-2 were generated by injecting recombinant human Sulf-2 protein into Sulf-2 null mice (see METHODS). Hybridomas were screened by ELISAs with the requirement that the supernatants react both with recombinant Sulf-2 and native Sulf-2 obtained from a cancer cell line (MCF7 cells). Three monoclonal antibodies, designated 8G1, 2E8, and 5D5, were characterized. They are all IgG class with the following isotypes: 8G1 (IgG₁), 2E8 (IgG_{2a}), and 5D5 (IgG_{2b}). The sequences of the variable regions of the heavy and light chains of these antibodies are shown in Figures 9-11. The purified antibodies all react with Sulf-2 protein by ELISA (Fig. 1). Two of the mAbs (8G1 and 5D5) react with the 75 kDa subunit of Sulf-2 and its 125 kDa proprotein precursor (but not Sulf-1) by western blotting (Fig. 2).

[00247] *Figure 1.* Sulf-2 mAbs react with native Sulf-2 derived from MCF7 cells. Conditioned medium from MCF7 cells was coated onto ELISA wells. Antibodies or irrelevant class-matched IgGs were reacted with the wells at the indicated concentrations. Reaction products were read at OD 405 nm.

[00248] *Figure 2.* 8G1 and 5D5 western blot of the 75 kDa subunit of recombinant and native Sulf-2. 30 μl of conditioned medium ("CM") (in OptiMEM) was obtained after transient transfection of HEK293 cells with HSulf-1 or HSulf-2 plasmids, or was obtained from MCF7 cells. The CM samples were run on a 7.5% SDS gel with reduction and transferred to a polyvinylidene fluoride (PVDF) membrane. The blot was probed with the indicated mAb or irrelevant mIgG₁ (control) at 2 $\mu\text{g/ml}$ in block for 1 hour at room temperature. After washing, the HRP-conjugated goat anti-mouse IgG was

added overnight at 4°C and bands were visualized using the enhanced chemiluminescence (ECL) system. Neither mAb reacts with Sulf-1.

[00249] *In vitro activities of the mAbs.* The three antibodies were tested for their abilities to revert the malignant phenotype of lung cancer cells (H292 cell line) in culture. Excessive cell growth is a characteristic of cancer cells (24). All three antibodies markedly slow the growth of these cells on plastic dishes (Fig. 3). The antibodies (8G1 and 5D5) recapitulated the effects of Sulf-2 knockdown on cell growth (Fig. 4), establishing that the antibodies were targeting Sulf-2 to produce a reduction in cell growth. The antibodies were tested in a “scratch wound assay” to determine effects on the enhanced cell migration of H292 cells. All three antibodies inhibited the migration of the lung cancer cells to the same extent as Sulf-2 knockdown (Fig. 5). Growth in soft-agar is a hallmark of malignant cells. All three antibodies also inhibited colony formation in soft agar (Fig. 6).

[00250] *Figure 3.* Sulf-2 mAbs slow cell growth of H292 cells. The indicated antibodies, class-matched irrelevant IgGs or PBS were added to cultures of H292 cells as described in Methods. Cell growth was monitored by the Cell Titer Blue as described in Methods. Means plus SD's based on 6 replicates are shown. Antibodies and control IgGs were added to a final concentration of 10 µg/ml. 2E8 differs from controls at 4 and 6 days of growth with a p value of <0.05. 8G1 and 5D5 differ from the controls at 4 and 6 days with a p value of <0.01.

[00251] *Figure 4.* Sulf-2 mAbs reduce cell growth of H292 to the same extent as Sulf-2 knockdown. H292 cells with Sulf-2 knockdown or mock knockdown (Control shRNA) were exposed to Sulf-2 mAbs (8G1 or 5D5), irrelevant class matched IgG's or PBS as described in Methods. Cell growth was monitored for 6 days. Values are means +SD's based on 6 replicates determinations. ** denotes p value of <0.01 for comparison with the respective controls.

[00252] *Figure 5.* Anti-Sulf-2 mAbs slow migration of H292 cells. The indicated mAbs, class-matched IgGs, or PBS (Control) was added to scratched monolayers of H292 cells as described in Methods. The mAbs and IgGs were added to a final concentration of 25 µg/ml. Gap sizes were measured at the different Means and SD's based on 4 replicates are shown. 5D5, 2E8, and 8G1 differ from the controls at 24 and 48 hours with a p value of <0.01.

[00253] *Figure 6.* Sulf-2 mAbs (anti-Sulf-2 mAbs) inhibit colony formation by H292 cells in agarose. The indicated Sulf-2 mAbs, class matched irrelevant IgGs, or PBS (Control) were added to cells in agarose. Colonies were counted after 21 days of culture. Antibodies and IgGs were added to a final concentration of 25 µg/ml. Shown are means +SDs which are based on 3 replicates per treatment. *denotes p<0.05 and ** denotes p<0.01 in comparisons with respective controls.

[00254] *In vivo activity of the mAbs.* The 8G1 mAb was tested for its effects on the growth of human lung cancer cells as xenograft tumors in immunocompromised host mice (nude mice). A pharmacokinetic study was first performed to establish an injection protocol which would maintain high levels of 8G1 in the blood of nude for the duration of the xenograft experiment. After subcutaneous injection of H292 cells, tumors were allowed to grow to a just-detectable size by

palpation. MAb 8G1, an irrelevant class-matched IgG₁ (IgG₁), or phosphate buffered saline (PBS) was injected intraperitoneally. Tumors size was monitored for three weeks. Upon termination, tumors were excised and weighed. 8G1 produced a marked inhibition of tumor size with time (Fig. 7A) and in the final tumor weight (Fig. 7B). These effects were seen relative to both PBS and the irrelevant IgG₁.

[00255] *Figure 7.* Nude mice were injected subcutaneously with H292 cells as described in Methods. The tumors were allowed to grown to a palpable size at which point mice were injected intraperitoneally thrice per week with 8G1, an isotype-matched irrelevant IgG₁, or PBS. A) Tumor volumes were measured at the indicated times. Means +SEMs based on 5 determinations are shown. *denotes $p < 0.05$ for comparisons between 8G1 and control IgG. B) Tumor weights were measured at the termination of the experiment. P values for comparisons are indicated.

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While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. An antibody that specifically binds an epitope in a sulfatase-2 polypeptide, wherein the antibody competes for binding to a Sulfatase-2 polypeptide with an antibody that comprises
 - (i) a V_LCDR1 comprising an amino acid sequence of SEQ ID NO:11, SEQ ID NO:17, or SEQ ID NO:23;
 - (ii) a V_LCDR2 comprising an amino acid sequence of SEQ ID NO:12, SEQ ID NO:18, or SEQ ID NO:24
 - (iii) a V_LCDR3 comprising an amino acid sequence of SEQ ID NO:13, SEQ ID NO:19, or SEQ ID NO:25;
 - (iv) a V_HCDR1 comprising an amino acid sequence of SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:20;
 - (v) a V_HCDR2 comprising an amino acid sequence of SEQ ID NO:9, SEQ ID NO:15, or SEQ ID NO:21; and
 - (vi) a V_HCDR3 comprising an amino acid sequence of SEQ ID NO:10, SEQ ID NO:16, or SEQ ID NO:22.
2. The antibody of claim 1, wherein the light chain variable region and the heavy chain variable region are present in separate polypeptides.
3. The antibody of claim 1, wherein the light chain variable region and the heavy chain variable region are present in a single polypeptide.
4. The antibody of claim 1, wherein the antibody binds the epitope with an affinity of from about 10⁷ M⁻¹ to about 10¹² M⁻¹.
5. The antibody of claim 1, wherein the heavy chain region is of the isotype IgG1, IgG2, IgG3, or IgG4.
6. The antibody of claim 1, wherein the antibody comprises a detectable label.
7. The antibody of claim 1, wherein the antibody is a Fv, scFv, Fab, F(ab')₂, or Fab'.
8. The antibody of claim 1, wherein the antibody comprises a covalently linked non-peptide synthetic polymer.

9. The antibody of claim 8, wherein the synthetic polymer is poly(ethylene glycol) polymer.
10. The antibody of claim 1, wherein the antibody comprises a covalently linked lipid or fatty acid moiety.
11. The antibody of claim 1, wherein the antibody comprises a covalently linked polysaccharide or carbohydrate moiety.
12. The antibody of claim 1, wherein the antibody comprises a contrast agent.
13. The antibody of claim 1, wherein the antibody comprises an affinity domain.
14. The antibody of claim 1, wherein the antibody is immobilized on a solid support.
15. The antibody of claim 1, wherein the antibody is a single chain Fv (scFv) antibody.
16. The antibody of claim 17, wherein the scFv is multimerized.
17. The antibody of claim 1, wherein the antibody comprises a polyamine modification.
18. The antibody of claim 1, wherein the antibody is a humanized antibody.
19. A recombinant expression vector comprising a nucleotide sequence encoding the antibody of claim 1, wherein the nucleotide sequence is operably linked to a transcriptional control element that is active in a eukaryotic cell.
20. A pharmaceutical composition comprising:
 - a) the antibody of claim 1; and
 - b) a pharmaceutically acceptable carrier.
21. The pharmaceutical composition of claim 20, wherein the antibody is encapsulated in a liposome.
22. A method of reducing tumor mass and/or cancer cell number in an individual having a cancer, the method comprising administering to the individual the pharmaceutical composition of claim 20.

23. The method of claim 22, wherein the cancer is non-small-cell lung cancer, hepatocellular carcinoma, pancreatic cancer, a central nervous system neoplasm, breast cancer, multiple myeloma, a head and neck cancer, gastric cancer, kidney cancer, or skin cancer.

24. A method of reducing proliferation of a cancer cell that overexpresses a sulfatase-2 polypeptide, the method comprising contacting the cell with the antibody of claim 1.

25. A method of treating dyslipoproteinemia associated with type 2 diabetes in an individual, the method comprising administering to the individual the pharmaceutical composition of claim 20.

26. A method of detecting a sulfatase-2 polypeptide in a biological sample obtained from an individual, the method comprising:

- a) contacting the biological sample with the antibody of claim 1; and
- b) detecting binding, if any, of the antibody to an epitope present in the sample.

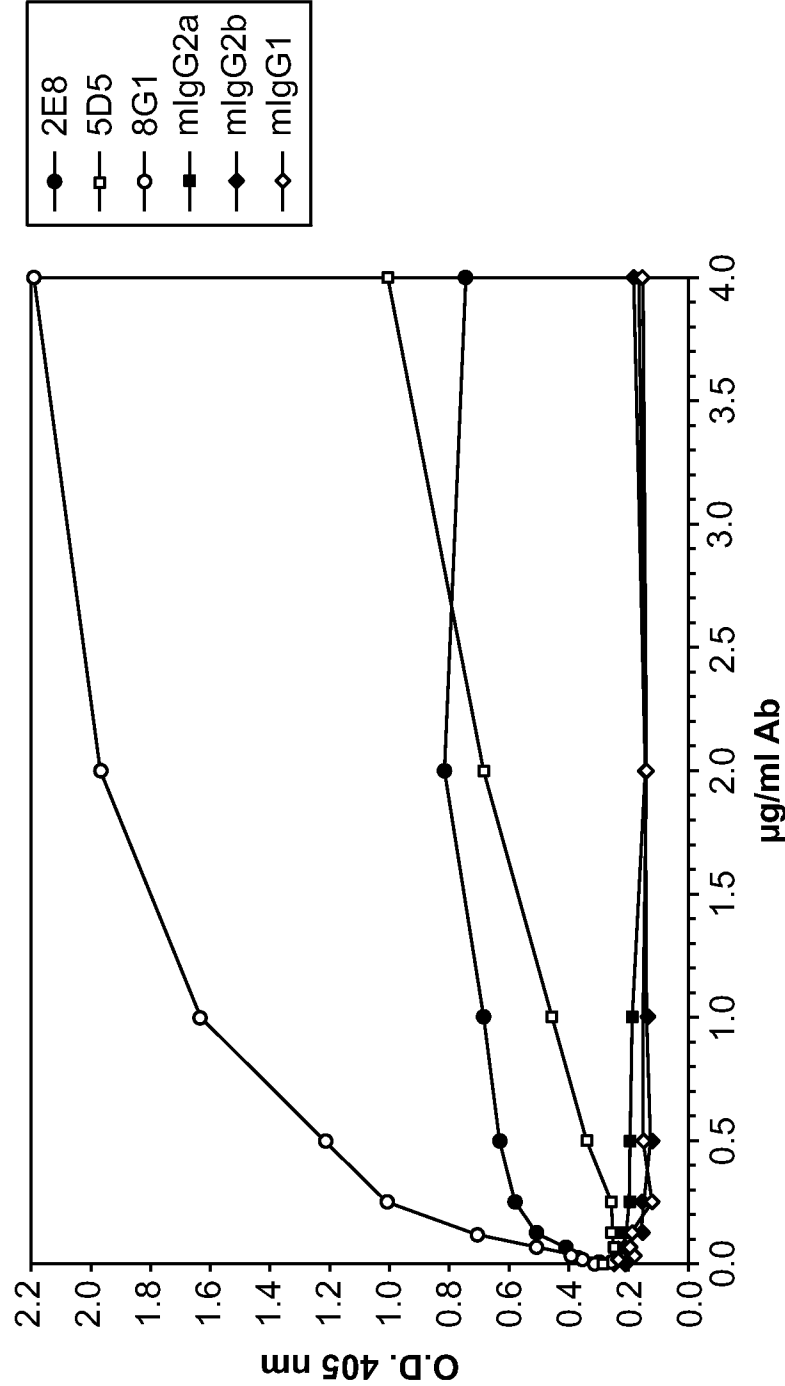


FIG. 1

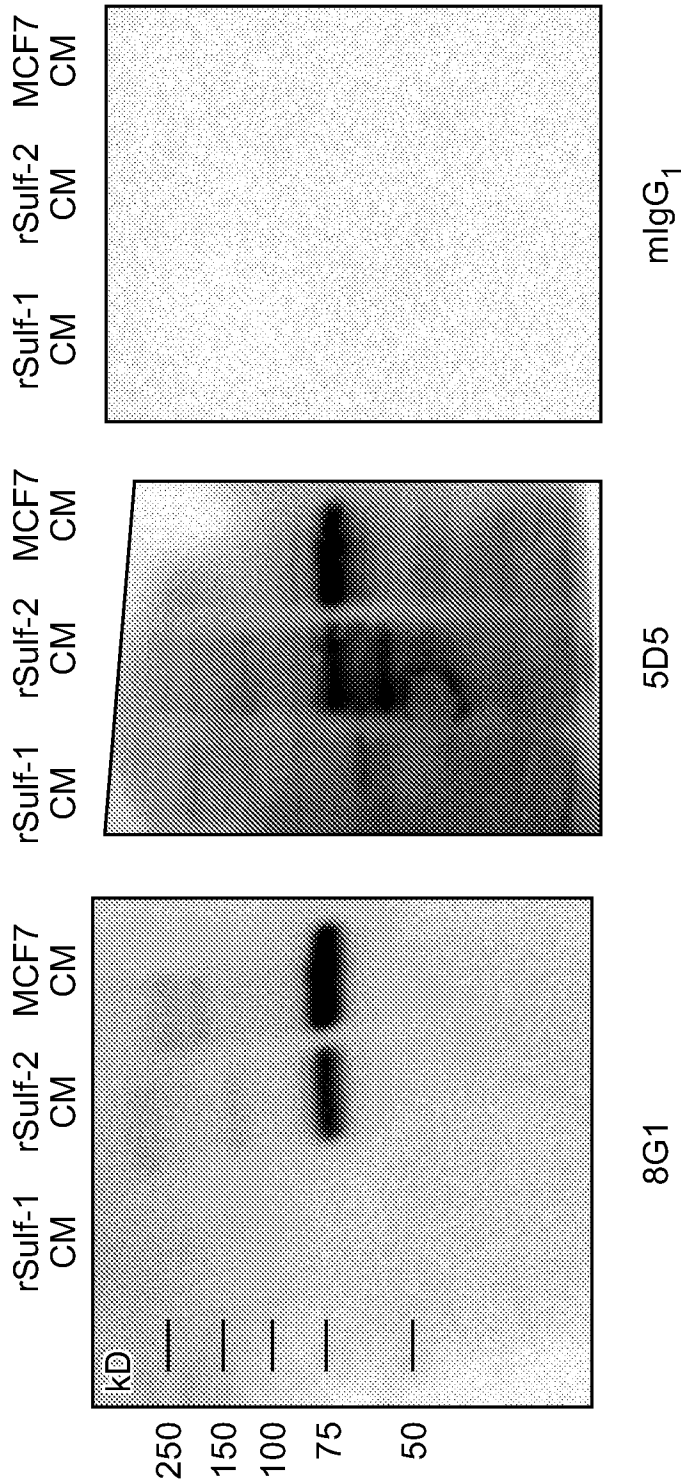


FIG. 2

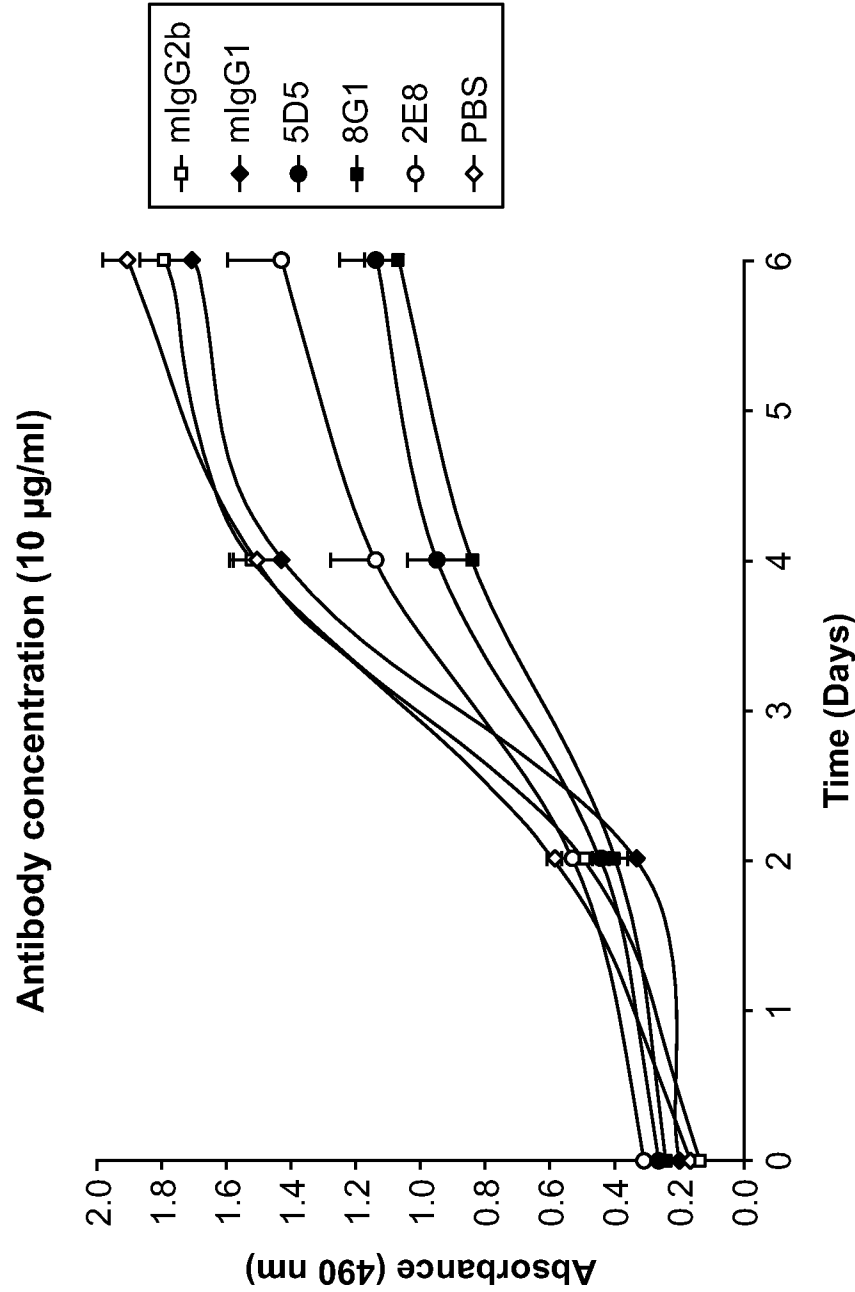


FIG. 3

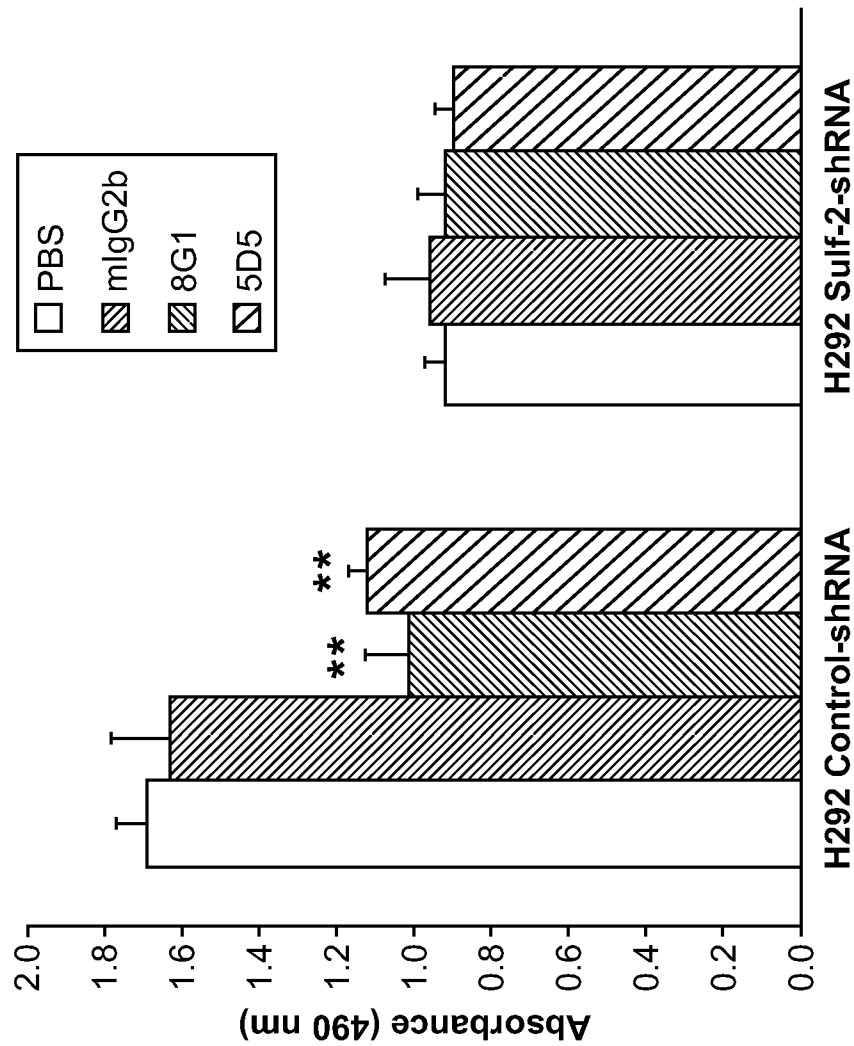


FIG. 4

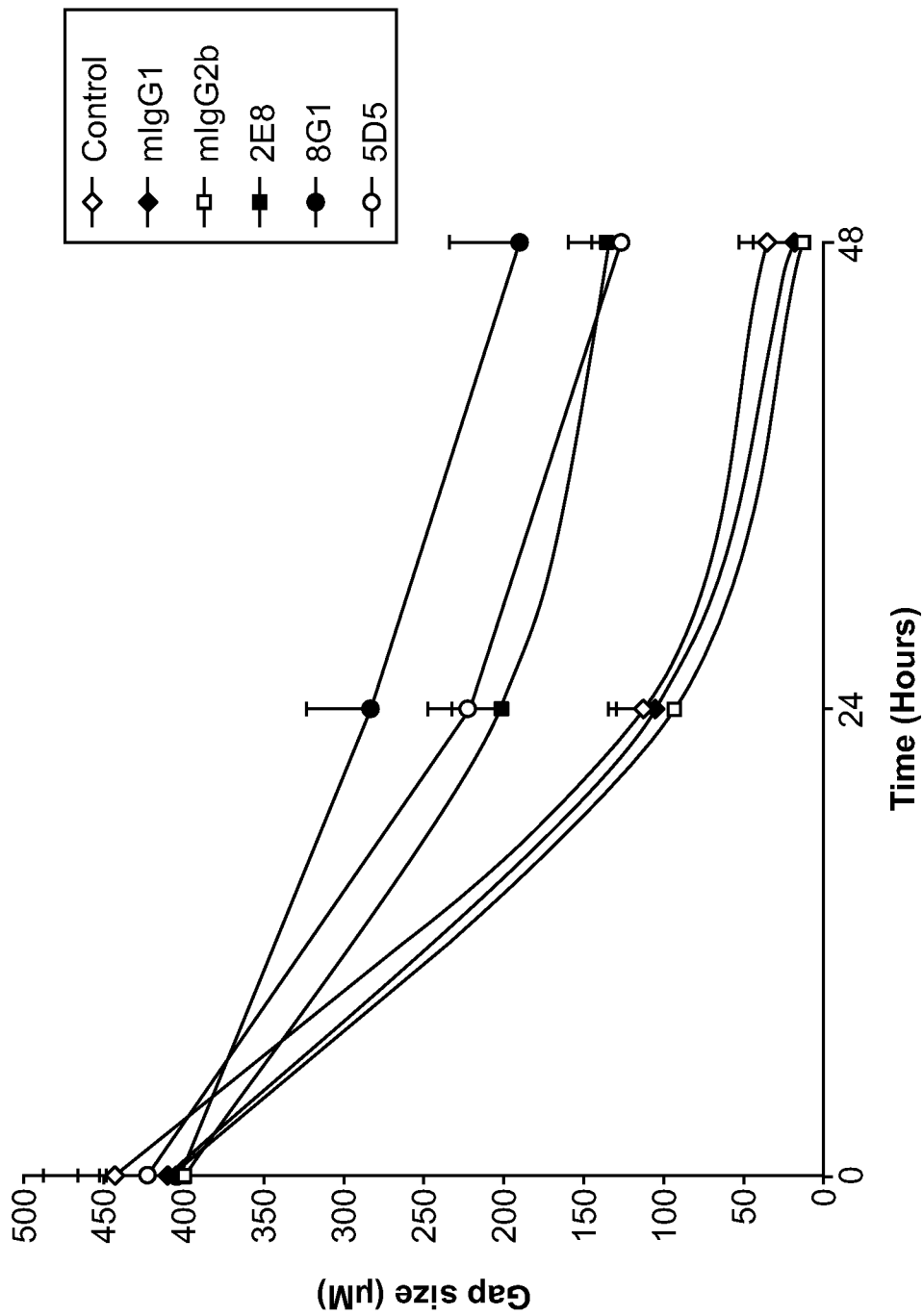


FIG. 5

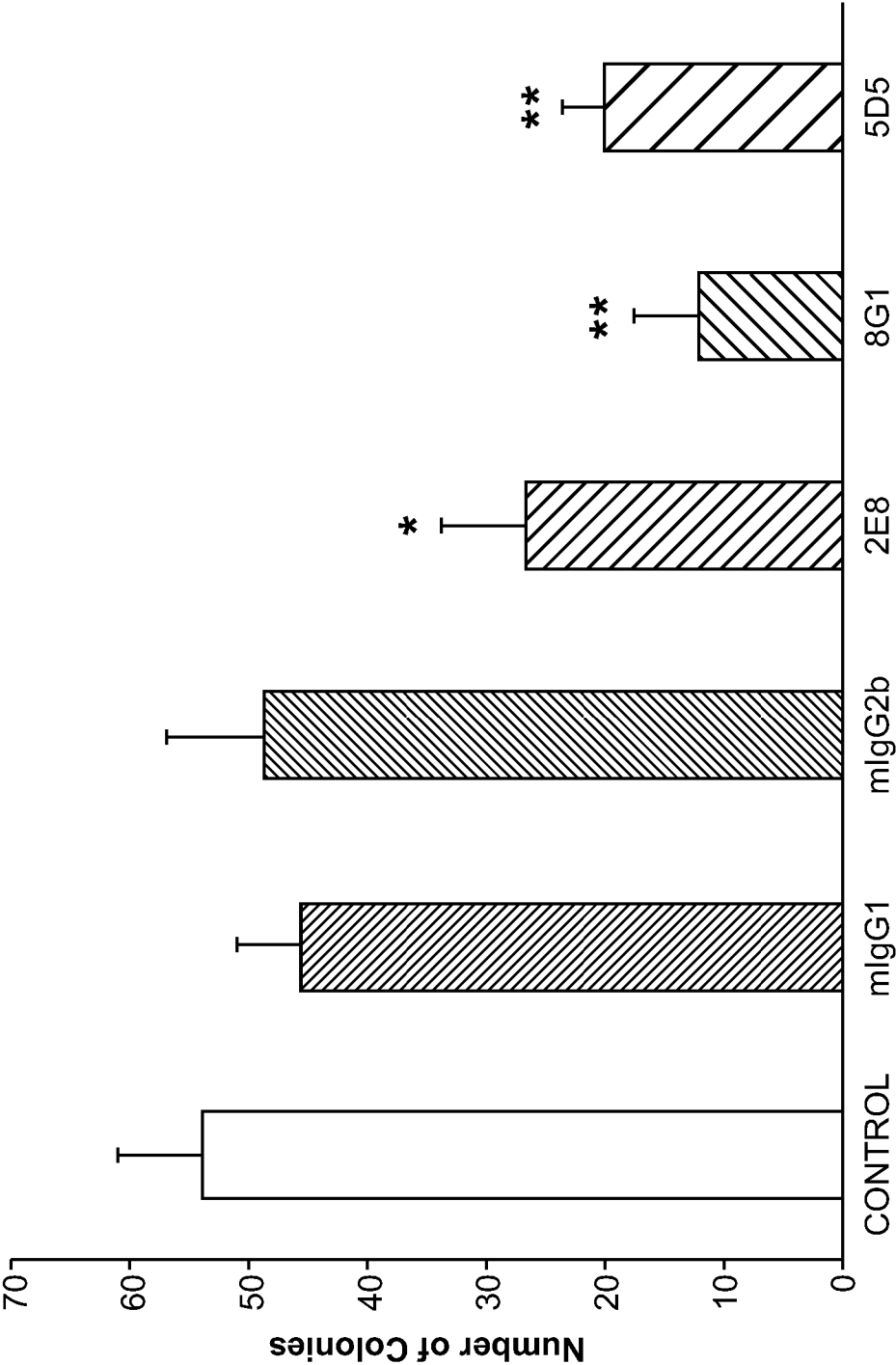


FIG. 6

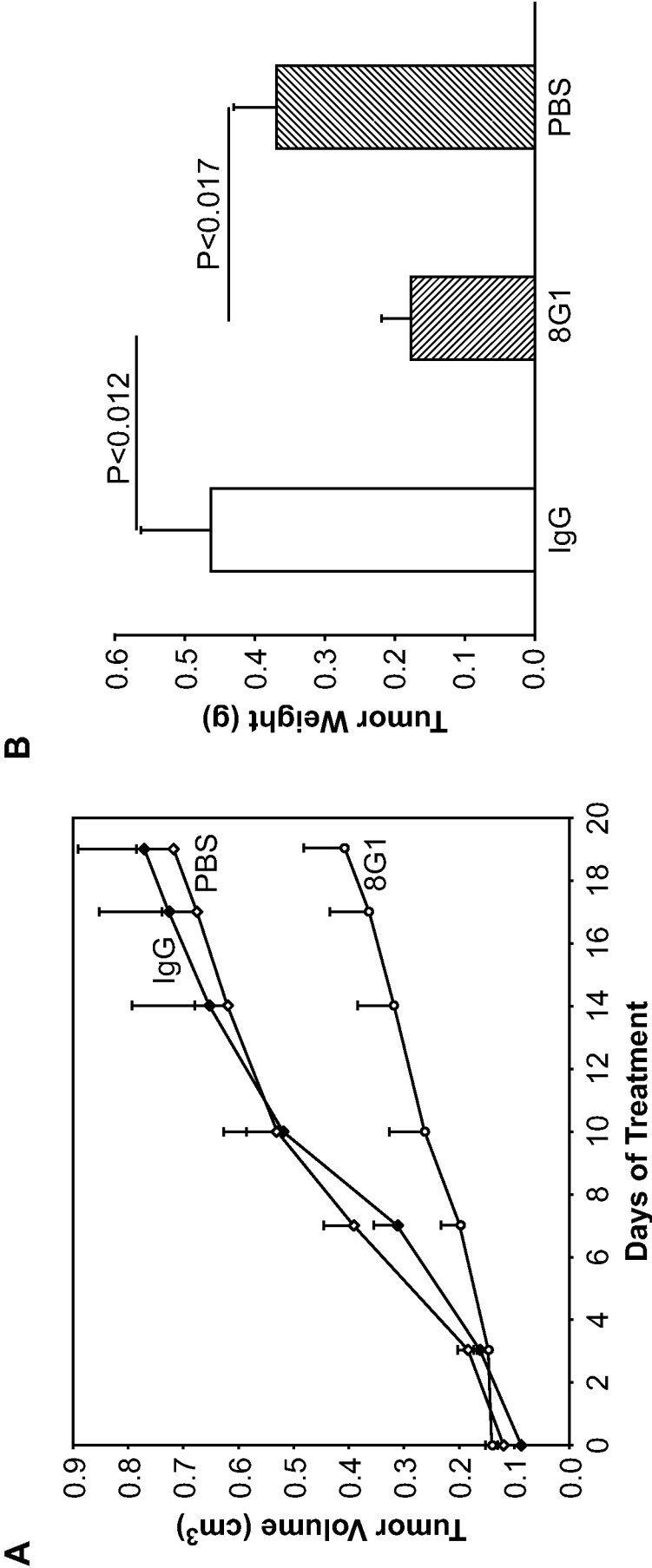


FIG. 7

***Homo sapiens* Sulfatase-2
GenBank NP_001155313**

```

1  mgppslvlcl lsatvflslg gssafllshhr lkgrfqrdr nirpniilvl tddqdvvelgs
61  mqvmnktrri meqggahfin afvttpmccp srssiltgky vhnhtytnn encsspswqa
121 qhesrtfavy lnstgyrtaf fgkylneyng syvppgwkw yphrpvlmvi shaaphgped sapqysrlfp
181 ekhgsdyskd yltlditnds vsffrtskkm yphrpvlmvi shaaphgped sapqysrlfp
241 nasqhityps nyapnpdkhw imrytgpmkp ihmeftnmlq rkrlqtlmsv ddsmetiynm
301 lvetgeldnt yivytadhgy higqfglvkg ksmpeyefdir vpfyvrgpvn eagclnphiv
361 lnidlaptil diagldipad mdgksilkl1 dterpvnrfh lkkmrvrwr sflvergkll
421 hkrndndkvda qeenflpkyq rvkdlcgrae yqtaceqlgq kwqcvedatg klklhkckgp
481 mrlggsrals nlvpkyygqg seactcdsgd yklslagrrk klfkkykas yvrssrsirsv
541 aievdgrvyh vglgdaaqpr nltkrhwpga pedqddkdg dfsgtgglpd ysaanpikvt
601 hrcyilendt vqcdldlyks lqawkdhklh idheietlqn kiknlrevrg hlkkkrpeec
661 dchkisyhtq hkgrlkhrgs slhpfrkgql ekdkvllre qkrkkklrk1 lkrlqnndtc
721 smpgltcfth dnqhwqtapf wtlgpfact sannntywcm rtinethnfl fcefatgfle
781 yfdlnstdpyq lmnvntldr dvlnqlhvql melrsckgyk qcnprtrnmd lgldkdggsye
841 qyrqfqrkw pemkrpssks lgqlwegweg (SEQ ID NO:1)

```

FIG. 8

8G1 - VH

QVKLEQSGPELVKPGASVRISCKASGYTFTTTYIHHWV/RQRPQQGLEWIGWIFPGNAIVKYDDNFKDKATLTADKSSSTAYIQLNSLKFE
 DSAVYFCAREEEANYDFNPMDYWGQGTSVSVSSAKTTPPSVYPLAPGSAAQTNMVTLGCLVK (SEQ ID NO:2)

V_H CDR1: GYTFTTTYIH (SEQ ID NO:8)

V_H CDR2: WIFPGNAIVKYDDNFKD (SEQ ID NO:9)

V_H CDR3: EEANYDFNPMDY (SEQ ID NO:10)

8G1-VL

DIVMTQTPSSLTVSVGEKVTMSCKSSOSLLYSGNOKNYLAWYQQKPGQSPQLLIYWASTRESGAPDRFTGSGSGTDFTLTISSVKAEDL
 AVYYCQQYFKYPYTFGGGTKLEIKRADAAPTVS (SEQ ID NO:3)

V_L CDR1: KSSQSLLYSGNQKNYLA (SEQ ID NO:11)

V_L CDR2: WASTRES (SEQ ID NO:12)

V_L CDR3: QQYFKYPYT (SEQ ID NO:13)

FIG. 9

10 / 11

5D5-VH**5D5-VH**

EVQLQESGSELSPGSSVKLSCKDFDDSEVFPIAYISWIRQKPGHGFEWIGDILPSIGSTIYGKKFEDKATLDADTVSNTAYLELDSLTSED
 SAIYYCAREEDYYDGRSSWGQGTTLTVSSAKTTPPSVYPLAPGCGD TTGSSVTLGCLVK (SEQ ID NO:4)

V_H CDR1: DSEVFPIAYIS (SEQ ID NO:14)

V_H CDR2: DILPSIGSTIYGKKFED (SEQ ID NO:15)

V_H CDR3: EDYYDGRSS (SEQ ID NO:16)

5D5-VL

DIVITQTTLSSLVSLGDQASISCRSSQSLVHTNGDITYLHHWYLQKPGQSPKLLIYKVSTRFAGVDPDRFSGSGGTDFTLKTSRVEAEDLGV
 YFCSQSTIYVPFTFGSGTKLEIKRADAAPTVS (SEQ ID NO:5)

V_L CDR1: RSSQSLVHTNGDITYLH (SEQ ID NO:17)

V_L CDR2: KVSTRFA (SEQ ID NO:18)

V_L CDR3: SQSTIYVPFT (SEQ ID NO:19)

FIG. 10

11 / 11

2E8-VH

QVQLQESGPGLVQPSQSLSTCTVSGFSLTSYGVHWWVRQSPGKGLEWLGVWWSGGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQADD
 TAIYYCARNGGLALDYWGQGTTLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVK (SEQ ID NO:6)

V_H CDR1: GFSLTSYGVH (SEQ ID NO:20)

V_H CDR2: VIWSGGSTDYNAAFIS (SEQ ID NO:21)

V_H CDR3: NGGLALDY (SEQ ID NO:22)

2E8-VL

DIVLTQSPAILSASPGEKVTMTCTCRASSVSVMHWWYQQKPGSSPKPWIVYVTSNLASGVPARFSGSGTSYSLTISRVEAEDAATYYCQQ
 WSSNPLTTFGAGTKLELKRADAAPTYS (SEQ ID NO:7)

V_L CDR1: RASSVSVMH (SEQ ID NO:23)

V_L CDR2: VTSNLAS (SEQ ID NO:24)

V_L CDR3: QQWSSNPLT (SEQ ID NO:25)

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/22855

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/00; C12N 15/63; A61K 39/395 (2012.01)

USPC - 530/388.1; 435/320.1; 424/146.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC - 530/388.1; 435/320.1; 424/146.1

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

USPC - 424/94.6 (text search, see terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(PGPB,USPT,EPAB,JPAB); Google Scholar (text search, see terms below)

Search Terms: Sulfatase 2, sulf 2, sulf2, hslf2, antibod\$, anti-sulf\$, epitope, seq, sequence, lipid, fatty acid, polyamine, covalent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0017022 A1 (NAWORTH et al.) 15 January 2009 (15.01.2009); Claims 24 and 27, Figure 7, paras [0055], [0067], [0068], [0127]-[0129], [0132], [0133], [0134], [0136], [0138], [0156], [0160] [0165], [0170], [0171], [0242]-[0244], [0252], [0303], [0331], [0333], [0350], [0374]	1-9, 11-16, 18-24, 26
Y		10, 17, 25
Y	Huang et al. Monoclonal antibody covalently coupled with fatty acid. JBC, 1980, Vol 255(17), pp 8015 - 8018; Abstract, (page 8015, col 1, para 1 - col 2, para 1)	10
Y	Poduslo et al. Polyamine modification increases the permeability of proteins at the blood-nerve and blood-brain barriers. J Neurochem, 1996, Vol 66(4), pp 1599 - 1609; Abstract only; [online] downloaded from http://www.ncbi.nlm.nih.gov/pubmed/8627316 on 5 May 2012	17
Y	Chen et al. Type 2 diabetes in mice induces hepatic overexpression of sulfatase 2, a novel factor that suppresses uptake of remnant lipoproteins. Hepatology, 2010, Vol 52, pp 1957 - 1967; Abstract, Discussion, Figure 2B, (page 1962, col 2, para 1)	25
A	Morimoto-Tomita et al. Sulf-2, a proangiogenic heparan sulfate endosulfatase, is upregulated in breast cancer. Neoplasia, 2005, vol 7(11), pp 1001-1010; (page 1002, col1, para 3)	1-26

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

30 April 2012 (30.04.2012)

Date of mailing of the international search report

30 MAY 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774