The invention provides SPARC binding antibodies that target disease sites, in particular, tumors and uses thereof to diagnose and treat diseases, in particular, cancerous tumors.
SPARC BINDING ANTIBODIES AND USES THEREOF

BACKGROUND OF THE INVENTION

[0001] Secreted Protein, Acidic, Rich in Cysteines (SPARC), also known as osteonectin, is a 281 amino acid glycoprotein. SPARC has affinity for a wide variety of ligands including cations (e.g., Ca²⁺, Cu²⁺, Fe²⁺), growth factors (e.g., platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF)), extracellular matrix (ECM) proteins (e.g., collagen I-V and collagen IX, vitronectin, and thrombospondin-1), endothelial cells, platelets, albumin, and hydroxyapatite. SPARC expression is developmentally regulated, and is predominantly expressed in tissues undergoing remodeling during normal development or in response to injury (see, e.g., Lane et al., *FASEB J.*, 8, 163-173 (1994)). High levels of SPARC protein are expressed in developing bones and teeth.

[0002] SPARC is a matricellular protein upregulated in several aggressive cancers, but is absent from the vast majority of normal tissues (Porter et al., *J. Histochem. Cytochem.*, 43, 791 (1995) and see below). Indeed, SPARC expression is induced among a variety of tumors (e.g., bladder, liver, ovary, kidney, gut, and breast). In bladder cancer, for example, SPARC expression has been associated with advanced carcinoma. Invasive bladder tumors of stage T2 or greater have been shown to express higher levels of SPARC than bladder tumors of stage T1 (or less superficial tumors), and have poorer prognosis (see, e.g., Yamanaka et al., *J. Urology*, 166, 2495-2499 (2001)). In meningiomas, SPARC expression has been associated with invasive tumors only (see, e.g., Rempel et al., *Clinical Cancer Res.*, 5, 237-241 (1999)). SPARC expression also has been detected in 74.5 % of in situ invasive breast carcinoma lesions (see, e.g., Bellahcene et al., *Am. J. Pathol.*, 146, 95-100 (1995)), and 54.2% of infiltrating ductal carcinoma of the breast (see, e.g., Kim et al., *J. Korean Med. Set.*, 13, 652-657 (1998)). SPARC expression also has been associated with frequent microcalcification in breast cancer (see, e.g., Bellahcene et al., *supra*), suggesting that SPARC expression may be responsible for the affinity of breast metastases for the bone. SPARC is also known to bind albumin (see, e.g., Schnitzer, *J. Biol. Chem.*, 269, 6072 (1994)).

[0003] Accordingly, there is a need for compositions and methods that take advantage of SPARC's role in disease and, in particular, SPARC's role in some cancers.
BRIEF SUMMARY OF THE INVENTION

[0004] In one aspect, the invention provides compositions comprising a SPARC binding antibody, wherein the SPARC binding antibody comprises Imm-2, Imm-3, or a combination thereof.

[0005] In another aspect, the invention provides methods of diagnosing or treating a disease, such as cancer, in an animal comprising administering a diagnostically or therapeutically effective amount of a composition comprising a SPARC binding antibody, wherein the SPARC binding antibody comprises Imm-2, Imm-3, or a combination thereof.

[0006] In all methods and compositions of the present invention, the SPARC binding antibody can be conjugated to a therapeutic or diagnostic active agent. Suitable animals for administration of the compositions provided by the invention and application of the methods of the invention include, without limitation, human patients.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0007] Figure 1 is a restriction map of pASK84 used for cloning and expression of the Fab regions of Imm-1 through Imm-12.

[0008] Figure 2 provides the amino acid sequences of two human anti-SPARC Fab clones Fab 6 and Fab 16 (SEQ ID NOs 15-16).

[0009] Figure 3 is a restriction map of the pBAD vector used for cloning and expression of Fab 16.

[0010] Figure 4 provides the amino acid sequences of Fabl6 in pBad (SEQ ID NO: 17).

[0011] Figure 5 provides amino acid sequences of framework regions (FWRs) and complementarity determining regions (CDRs) for Imm1 (SEQ ID NOs 1 and 8), Imm2 (SEQ ID NOs 2 and 9), Imm3 (SEQ ID NOs 3 and 10), Imm4 (SEQ ID NOs 4 and 11), Imm6 (SEQ ID NOs 5 and 12), Imm1O(SEQ ID NOs 6 and 13), and Imm12 (SEQ ID NOs 7 and 14).

[0012] Figure 6 provides quantitative ELISA results of 1:1, 1:10, and 1:100 dilutions of Imm1-6 and Imm8-12 supernatants against human SPARC, as well as a control mAb.

[0013] Figure 7 provides quantitative ELISA results of 0.04 µg/mL, 0.2 µg/mL, 1 µg/mL, and 5 µg/mL concentrations of purified Imm1-12 antibodies against human SPARC, as well as positive and negative controls.
Figure 8 provides quantitative ELISA results comparing the binding of Imm1, Imm3, Imm4, Imm7, Imm9, and Imm10 antibodies to HTI-SPARC (platelet SPARC) and binding of Imm10, Imm1, Imm12, and control antibodies to Biol-SPARC.

Figure 9 provides quantitative ELISA results of Fab 16 (SEQ ID NO: 20) binding to HTI-SPARC (platelet SPARC) and Biol-SPARC at various concentrations.

Figure 10 is a sensorgram prepared using surface plasmon resonance of Fab 16 (SEQ ID NO: 20) binding to human HTI SPARC.

Figure 11 is a sensorgram prepared using surface plasmon resonance of Fab 16 (SEQ ID NO: 20) binding to human Biol SPARC.

Figure 12 provides quantitative ELISA results of Imm1, Imm2, Imm3, and Imm14 binding against human SPARC at various concentrations.

Figure 13 is a Western Blot of denatured Imm-series antibodies against human SPARC.

Figure 14 depicts epitope mapping of Imm1, Imm2, Imm3, Imm4, Imm7, Imm9, Imm10, Imm1, Imm2, Imm3, Imm4, and control mAbs.

Figure 15 provides peptide sequences from a phage display library against Imm1, Imm2, Imm3, Imm4, Imm7, and control antibodies, aligned against SPARC.

Figure 16 provides peptide sequences from a phage display library against Imm9, Imm10, Imm1, Imm2, Imm3, and Imm4, aligned against SPARC.

Figure 17 depicts in vivo tumor localization of Imm1, Imm2, Imm3, Imm4, Imm7, Imm9, Imm10, Imm1, Imm2, Imm3, Imm4, and control antibodies over 36 days.

Figure 18 is a photograph of exemplary nude mice demonstrating visualization of tumors in vivo via Imm2 at day 18.

Figure 19 is a photograph of exemplary nude mice demonstrating visualization of tumors in vivo via Imm2 at day 28.

Figure 20 depicts the effect of anti-SPARC antibodies Imm2, Imm12, and Imm14, as well as control mlgG, on survival of animals bearing LL/2 Lewis Lung Carcinoma.

Figure 21 is a restriction map of the pcDNA3002NEO vector used for the cloning and expression of fully-human antibodies Imm13 and Imm14 from Fab6 (SEQ ID NO: 15) and Fab16 (SEQ ID NO: 16).
DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention relates to the Imm-series of antibodies, a series of 14 SPARC binding antibodies which was analyzed for binding activity and tumor specificity. Surprisingly, the analysis revealed that although all of the antibodies bound SPARC in screening ELISA, two of the antibodies, Imm-2, Imm-3, can be used particularly effectively to target tumors. Without being bound by any particular theories, it is postulated that these two tumor-targeting antibodies are directed to different SPARC epitopes than the other Imm-series antibodies.

[0029] Definitions

[0030] "Peptide" and "polypeptide" are used interchangeably herein and refer to a compound made up of a chain of amino acid residues linked by peptide bonds. An "active portion" of a polypeptide means a peptide that is less than the full length polypeptide, but which retains measurable biological activity and retains biological detection.

[0031] As used herein, the term "tumor" refers to any neoplastic growth, proliferation or cell mass whether benign or malignant (cancerous), whether a primary site lesion or metastases.

[0032] As used herein, the term "cancer" refers to a proliferative disorder caused or characterized by a proliferation of cells which have lost susceptibility to normal growth control. Cancers of the same tissue type usually originate in the same tissue, and may be divided into different subtypes based on their biological characteristics. Four general categories of cancer are carcinoma (epithelial cell derived), sarcoma (connective tissue or mesodermal derived), leukemia (blood-forming tissue derived) and lymphoma (lymph tissue derived). Over 200 different types of cancers are known, and every organ and tissue of the body may be affected. Specific examples of cancers that do not limit the definition of cancer may include melanoma, leukemia, astrocytoma, glioblastoma, retinoblastoma, lymphoma, glioma, Hodgkin's lymphoma, and chronic lymphocytic leukemia. Examples of organs and tissues that may be affected by various cancers include pancreas, breast, thyroid, ovary, uterus, testis, prostate, pituitary gland, adrenal gland, kidney, stomach, esophagus, rectum, small intestine, colon, liver, gall bladder, head and neck, tongue, mouth, eye and orbit, bone, joints, brain, nervous system, skin, blood, nasopharyngeal tissue, lung, larynx, urinary tract, cervix, vagina, exocrine glands, and endocrine glands. Alternatively, a cancer can be multicentric or of unknown primary site (CUPS).
As used herein "a suitable anti-SPARC antibody" or "a SPARC binding antibody" refers to a tumor targeting antibody capable of binding to SPARC with specificity.

As used herein "tumor targeting antibody" refers to a disease targeting antibody wherein the disease is a tumor, cancer, neoplasm or the like.

As used herein "a disease targeting antibody" refers to an antibody that increases the accumulation of an agent at a disease site, in particular, at a tumor site by at least 25%, more preferably at least 50%, even more preferably at least 75%, even more preferably at least 100%, even more preferably at least 3 fold, even more preferably at least 5 fold, even more preferably at least 10 fold, even more preferably at least 20 fold, and most preferably at least 100 fold, as determined by any suitable conventional imaging technique or biopsy and chemical analysis.

As used herein "therapeutically effective amount" refers to an amount of a composition that relieves (to some extent, as judged by a skilled medical practitioner) one or more symptoms of the disease or condition in a mammal. Additionally, by "therapeutically effective amount" of a composition is meant an amount that returns to normal, either partially or completely, physiological or biochemical parameters associated with or causative of a disease or condition. A clinician skilled in the art can determine the therapeutically effective amount of a composition in order to treat or prevent a particular disease condition, or disorder when it is administered, such as intravenously, subcutaneously, intraperitoneally, orally, or through inhalation. The precise amount of the composition required to be therapeutically effective will depend upon numerous factors, e.g., such as the specific activity of the active agent, the delivery device employed, physical characteristics of the agent, purpose for the administration, in addition to many patient specific considerations. But a determination of a therapeutically effective amount is within the skill of an ordinarily skilled clinician upon the appreciation of the disclosure set forth herein.

The terms "treating," "treatment," "therapy," and "therapeutic treatment" as used herein refer to curative therapy, prophylactic therapy, or preventative therapy. An example of "preventative therapy" is the prevention or lessening the chance of a targeted disease (e.g., cancer or other proliferative disease) or related condition thereto. Those in need of treatment include those already with the disease or condition as well as those prone to have the disease or condition to be prevented. The terms "treating," "treatment," "therapy," and "therapeutic treatment" as used herein also describe the management and care of a mammal for the purpose of combating a disease, or related condition, and includes the administration of a
composition to alleviate the symptoms, side effects, or other complications of the disease, condition. Therapeutic treatment for cancer includes, but is not limited to, surgery, chemotherapy, radiation therapy, gene therapy, and immunotherapy.

[0038] As used herein, the term "agent" or "drug" or "therapeutic agent" refers to a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues that are suspected of having therapeutic properties. The agent or drug can be purified, substantially purified or partially purified. An "agent" according to the present invention, also includes a radiation therapy agent or a "chemotherapeutic agent."

[0039] As used herein, the term "diagnostic agent" refers to any chemical used in the imaging of diseased tissue, such as, e.g., a tumor.

[0040] As used herein, the term "chemotherapeutic agent" refers to an agent with activity against cancer, neoplastic, and/or proliferative diseases.

[0041] As used herein, the term "radiotherapeutic regimen" or "radiotherapy" refers to the administration of radiation to kill cancerous cells. Radiation interacts with various molecules within the cell, but the primary target, which results in cell death is the deoxyribonucleic acid (DNA). However, radiotherapy often also results in damage to the cellular and nuclear membranes and other organelles. DNA damage usually involves single and double strand breaks in the sugar-phosphate backbone. Furthermore, there can be cross-linking of DNA and proteins, which can disrupt cell function. Depending on the radiation type, the mechanism of DNA damage may vary as does the relative biologic effectiveness. For example, heavy particles (i.e. protons, neutrons) damage DNA directly and have a greater relative biologic effectiveness. Whereas, electromagnetic radiation results in indirect ionization acting through short-lived, hydroxyl free radicals produced primarily by the ionization of cellular water. Clinical applications of radiation consist of external beam radiation (from an outside source) and brachytherapy (using a source of radiation implanted or inserted into the patient). External beam radiation consists of X-rays and/or gamma rays, while brachytherapy employs radioactive nuclei that decay and emit alpha particles, or beta particles along with a gamma ray.

[0042] As used herein the term "alternative therapeutic regimen" or "alternative therapy" (not a first line chemotherapeutic regimen as described above) may include for example, receptor tyrosine kinase inhibitors (for example Iressa™ (gefitinib), Tarceva™ (erlotinib), Erbitux™ (cetuximab), imatinib mesilate (Gleevec™), proteosome inhibitors (for example
bortezomib, Velcade™); VEGFR2 inhibitors such as PTK787 (ZK222584), aurora kinase inhibitors (for example ZM447439); mammalian target of rapamycin (mTOR) inhibitors, cyclooxygenase-2 (COX-2) inhibitors, rapamycin inhibitors (for example sirolimus, Rapamune™); farnesyltransferase inhibitors (for example tipifarnib, Zarnestra); matrix metalloproteinase inhibitors (for example BAY 12-9566; sulfated polysaccharide tecogalan); angiogenesis inhibitors (for example Avastin™ (bevacizumab); analogues of fumagillin such as TNP-4; carboxyaminotriazole; BB-94 and BB-2516; thalidomide; interleukin-12; linomide; peptide fragments; and antibodies to vascular growth factors and vascular growth factor receptors); platelet derived growth factor receptor inhibitors, protein kinase C inhibitors, mitogen-activated kinase inhibitors, mitogen-activated protein kinase kinase inhibitors, Rouse sarcoma virus transforming oncogene (SRC) inhibitors, histonedeacetylase inhibitors, small hypoxia-inducible factor inhibitors, hedgehog inhibitors, and TGF-β signalling inhibitors. Furthermore, an immunotherapeutic agent would also be considered an alternative therapeutic regimen. For example, serum or gamma globulin containing preformed antibodies; nonspecific immunostimulating adjuvants; active specific immunotherapy; and adoptive immunotherapy. In addition, alternative therapies may include other biological-based chemical entities such as polynucleotides, including antisense molecules, polypeptides, antibodies, gene therapy vectors and the like. Such alternative therapeutics may be administered alone or in combination, or in combination with other therapeutic regimens described herein. Methods of use of chemotherapeutic agents and other agents used in alternative therapeutic regimens in combination therapies, including dosing and administration regimens, will also be known to a one skilled in the art.

[0043] **Antibodies**

[0044] The invention provides a SPARC binding antibody. In particular, the SPARC binding antibody can be Imm-2, Imm-3, or combinations thereof.

[0045] In addition, the invention provides for a SPARC binding antibody capable of binding both SPARC found in the blood, e.g. HTI (platelet) SPARC and SPARC found at a tumor site, e.g. Biol -SPARC. Various methods of determining antibody binding strength are known to those of ordinary skill in the art.

[0046] For human use, in order to avoid immunogenicity and immune response, it is preferable to use a humanized SPARC binding antibody or suitable fragments such as Fab’, Fab, or Fab2. Humanized antibody or fragments thereof can be produced, for example, using one of the following established methods: 1) a humanized antibody can be constructed using
human IgG backbone replacing the variable CDR region with that of an antibody against SPARC, where the heavy and light chain are independently expressed under separate promoters or coexpressed under one promoter with an IRES sequence; 2) a humanized monoclonal antibody can be raised against SPARC using a mouse engineered to have a human immune system; 3) a humanized antibody against SPARC can be raised using phagemid (MI 3, lambda coliphage, or any phage system capable of surface presentation). To construct the full length antibody, the variable region can be transferred onto the CDR of both a heavy chain and a light chain. The coexpression of the heavy chain and light chain in mammalian cells such as CHO, 293, or human myeloid cells can provide a full length antibody. Similarly, Fab', Fab, or Fab2 fragments and single chain antibodies can be prepared using well established methods.

[0047] The SPARC binding antibodies of the present invention include whole antibodies as well as fragments of the antibody retaining the binding site for SPARC (e.g., Fab', Fab and Fab2). The antibody can be any class of antibody, e.g., IgM, IgA, IgG, IgE, IgD, and IgY. The antibody can be, for example, a divalent, monovalent, or chimeric antibody with one valence for SPARC and another for an active agent (such as rTF or ricin A, or another active agent as described herein). The humanized antibody is not limited to IgG. The same technologies can be used to generate all other classes of antibodies such as IgE, IgA, IgD, IgM, each having different antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) activities appropriate to particular disease target. Functional fragments of the antibody can be generated by limited proteolysis. These fragments can be monovalent such as Fab' or divalent, such as Fab2. Fragments can also be synthesized as single chain scFv or diabodies in E. coli.

[0048] Compositions

[0049] The invention provides a composition comprising a SPARC binding antibody as described above. In some embodiments, the composition comprises either Imm-2 or Imm-3 along with a suitable carrier. In other embodiments, the composition comprises a combination of Imm-2 and Imm-3 along with a suitable carrier. In preferred embodiments, the composition is a pharmaceutically acceptable composition comprising a SPARC binding antibody and a pharmaceutically acceptable carrier.

[0050] The compositions of the present invention can further comprise an active agent. In some embodiments, the active agent is a pharmaceutically active therapeutic agent directly able to exert its pharmacological effect. In other embodiments, the active agent is a
diagnostic agent. In preferred embodiments, the active agent is a diagnostic or therapeutic active agent conjugated to a tumor-targeting SPARC binding antibody. It will be understood that some active agents are useful as both diagnostic and therapeutic agents, and therefore such terms are not mutually exclusive.

[0051] Compositions of the present invention can be used to enhance delivery of the active agent to a disease site relative to delivery of the active agent alone, or to enhance SPARC clearance resulting in a decrease in blood level of SPARC. In preferred embodiments, the decrease in blood level of SPARC is at least about 10%. In more preferred embodiments, the decrease in blood level of SPARC is at least about 15%, 20%, 25%, 30%, 35%, 40%, 45%, or, most preferably, at least about 50%.

[0052] The active agent can be any suitable therapeutic agent or diagnostic agent, such as a chemotherapeutic or anticancer agent. Suitable chemotherapeutic agents or other anticancer agents for use in accordance with the invention include, but are not limited to, tyrosine kinase inhibitors (genistein), biologically active agents (TNF, tTF), radionuclides (131I, 90Y, 111In, 211At, 32P and other known therapeutic radionuclides), adriamycin, ansamycin antibiotics, asparaginase, bleomycin, busulphan, cisplatin, carboplatin, carmustine, capecitabine, chlorambucil, cytarabine, cyclophosphamide, camptothecin, dacarbazine, dactinomycin, daunorubicin, dexrazoxane, docetaxel, doxorubicin, etoposide, epothilones, floxuridine, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine, mechlorethamine, mercaptopurine, mephalan, methotrexate, rapamycin (sirolimus) and derivatives, mitomycin, mitotane, mitoxantrone, nitrosurea, paclitaxel, pamidronate, pentostatin, plicamycin, procarbazine, rituximab, streptozocin, teniposide, thioguanine, thiopeta, taxanes, vinblastine, vincristine, vinorelbine, taxol, combretastatins, discodermolides, and transplatinum.

[0053] Other suitable chemotherapeutic agents for use in accordance with invention include, without limitation, antimitabolites (e.g., asparaginase), antimitotics (e.g., vinca alkaloids), DNA damaging agents (e.g., cisplatin), proapoptotics (agents which induce programmed-cell-death or apoptosis) (e.g. epipodophylotoxins), differentiation inducing agents (e.g., retinoids), antibiotics (e.g., bleomycin), and hormones (e.g., tamoxifen, diethylstibestrol). Further, suitable chemotherapeutic agents for use in accordance with the invention include antiangiogenesis agents (angiogenesis inhibitors) such as, e.g., INF-alpha, fumagillin, angiostatin, endostatin, thalidomide, and the like.
Preferred chemotherapeutic agents include docetaxel, paclitaxel, and combinations thereof. "Combinations thereof refers to both the administration of dosage forms including more than one drug, for example, docetaxel and paclitaxel, as well as the sequential but, temporally distinct, administration of docetaxel and paclitaxel (e.g., the use of docetaxel in one cycle and paclitaxel in the next). Particularly preferred chemotherapeutic agents comprise particles of protein-bound drug, including but not limited to, wherein the protein making up the protein-bound drug particles comprises albumin including wherein more than 50% of the chemotherapeutic agent is in nanoparticle form. Most preferably the chemotherapeutic agent comprises particles of albumin-bound paclitaxel, such as, e.g., Abraxane®. Such albumin-bound paclitaxel formulations can be used in accordance with the invention where the paclitaxel dose administered is from about 30 mg/m2 to about 1000 mg/m2 with a dosing cycle of about 3 weeks (i.e., administration of the paclitaxel dose once every about three weeks). Further, it is desirable that the paclitaxel dose administered is from about 50 mg/m2 to about 800 mg/m2, preferably from about 80 mg/m2 to about 700 mg/m2, and most preferably from about 250 mg/m2 to about 300 mg/m2 with a dosing cycle of about 3 weeks.

Other therapeutic agents also include, without limitation, biologically active polypeptides, antibodies and fragments thereof, lectins, and toxins (such as ricin A), or radionuclides. Suitable antibodies for use as active agents in accordance with the invention include, without limitation, conjugated (coupled) or unconjugated (uncoupled) antibodies, monoclonal or polyclonal antibodies, humanized or unhumanized antibodies, as well as Fab', Fab, or Fab2 fragments, single chain antibodies and the like. Contemplated antibodies or antibody fragments can be Fc fragments of IgG, IgA, IgD, IgE, or IgM. In various preferred embodiments, the active agent is the Fc fragment of the antibody itself, a single chain antibody, a Fab fragment, diabody, and the like. In more preferred embodiments, the antibody or antibody fragment mediates complement activation, cell mediated cytotoxicity, and/or opsonization.

In addition, the pharmaceutically active agent can be an siRNA. In preferred embodiments, the siRNA molecule inhibits expression of an gene associated with tumors such as, for example, c-Sis and other growth factors, EGFR, PDGFR, VEGFR, HER2, other receptor tyrosine kinases, Src-family genes, Syk-ZAP-70 family genes, BTK family genes, other cytoplasmic tyrosine kinases, Raf kinase, cyclin dependent kinases, other cytoplasmic serine/threonine kinases, Ras protein and other regulatory GTPases.
[0057] The invention further provides a diagnostic agent conjugated to a SPARC binding antibody. Suitable diagnostic agents include, e.g., fluorochromes, radioisotopes or radionuclides, MRI contrast agents, X-ray contrast agents, ultrasound contrast agents and PET contrast agents.

[0058] The active agent can be coupled to the tumor-targeting SPARC binding antibody using any method known to one of skill in the art. For example, the SPARC binding antibody and the active agent can be coupled using a method such as biotin-streptavidin conjugation, chemical conjugation, covalent coupling, antibody coupling, and/or direct expression (e.g., a chimeric protein).

[0059] In other embodiments, free amino groups in SPARC binding antibodies can be conjugated with reagents such as carbodiimides or heterobifunctional agents. In addition, sugar moieties bound to suitable SPARC binding antibodies, can be oxidized to form aldehyde groups useful in a number of coupling procedures known in the art. The conjugates formed in accordance with the invention can be stable, in vivo, or labile, such as enzymatically degradeable tetrapeptide linkages, or acid-labile, cis-aconityl, or hydrazone linkages.

[0060] SPARC binding antibodies can also be conjugated to polyethylene glycol (PEG). PEG conjugation can increase the circulating half-life of a protein, reduce the protein's immunogenicity and antigenicity, and improve the bioactivity. Any suitable method of conjugation can be used, including but not limited to, e.g., reacting methoxy-PEG with a SPARC binding antibody's available amino groups or other reactive sites such as, e.g., histidines or cysteines. In addition, recombinant DNA approaches can be used to add amino acids with PEG-reactive groups to the inventive SPARC binding antibodies. PEG can be processed prior to reacting it with a SPARC binding antibody, e.g., linker groups can be added to the PEG. Further, releasable and hybrid PEG-ylation strategies can be used in accordance with the invention, such as, e.g., the PEG-ylation of a SPARC binding antibody such that the PEG molecules added to certain sites in the SPARC binding antibody are released in vivo. Such PEG conjugation methods are known in the art (See, e.g., Greenwald et al., Adv. Drug Delivery Rev. 55:217-250 (2003)).

[0061] Contemplated SPARC binding antibodies and conjugates thereof can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or
such as organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0062] The compositions of the present inventions are generally provided in a formulation with a carrier, such as a pharmaceutically acceptable carrier. Typically, the carrier will be liquid, but also can be solid, or a combination of liquid and solid components. The carrier desirably is a physiologically acceptable (e.g., a pharmaceutically or pharmacologically acceptable) carrier (e.g., excipient or diluent). Suitable pharmaceutical excipients include stabilizers, antioxidants, osmolality adjusting agents, buffers, and pH adjusting agents. Suitable additives include physiologically biocompatible buffers, additions of chelants or calcium chelate complexes, or, optionally, additions of calcium or sodium salts. Pharmaceutical compositions can be packaged for use in liquid form, or can be lyophilized. Preferred physiologically acceptable carrier media are water, buffered water, normal saline, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. Physiologically acceptable carriers are well known and are readily available. The choice of carrier will be determined, at least in part, by the location of the target tissue and/or cells, and the particular method used to administer the composition.

[0063] The composition can be formulated for administration by a route including intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal, epidural, topical, percutaneous, subcutaneous, transmucosal (including, for example, pulmonary), intranasal, rectal, vaginal, or oral. The composition also can comprise additional components such as diluents, adjuvants, excipients, preservatives, and pH adjusting agents, and the like.

[0064] Formulations suitable for injectable administration include aqueous and nonaqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, lyoprotectants, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, or tablets.
Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Preferably solutions for injection are free of endotoxin. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. In all cases, the formulation must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxycellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

In preferred embodiments, the active ingredients can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Specifically, liposomes containing the SPARC binding antibodies can be prepared by such methods as described in Rezler et al., J. Am. Chem. Soc. 129(16): 4961-72 (2007); Samad et al., Curr. Drug Deliv. 4(4): 297-305 (2007); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556.

Particularly useful liposomes can be generated by, for example, the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Polypeptides of the
The present invention can be conjugated to the liposomes as described in Werle et al., *Int. J. Pharm.* 370(1-2): 26-32 (2009).

[0068] In other embodiments, a composition can be delivered using a natural virus or virus-like particle, a dendrimer, carbon nanoassembly, a polymer carrier, a paramagnetic particle, a ferromagnetic particle, a polymersome, a filomicelle, a micelle or a lipoprotein.

[0069] Administration into the airways can provide either systemic or local administration, for example to the trachea and/or the lungs. Such administration can be made via inhalation or via physical application, using aerosols, solutions, and devices such as a bronchoscope. For inhalation, the compositions herein are conveniently delivered from an insufflator, a nebulizer, a pump, a pressurized pack, or other convenient means of delivering an aerosol, non-aerosol spray of a powder, or non-aerosol spray of a liquid. Pressurized packs can comprise a suitable propellant such as a liquefied gas or a compressed gas. Liquefied gases include, for example, fluorinated chlorinated hydrocarbons, hydrochlorofluorocarbons, hydrochlorocarbons, hydrocarbons, and hydrocarbon ethers. Compressed gases include, for example, nitrogen, nitrous oxide, and carbon dioxide. In particular, the use of dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas is contemplated. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a controlled amount. In administering a dry powder composition, the powder mix can include a suitable powder base such as lactose or starch. The powder composition can be presented in unit dosage form such as, for example, capsules, cartridges, or blister packs from which the powder can be administered with the aid of an inhalator or insufflator.

[0070] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays, inhaled aerosols, rectal or vaginal suppositories, mouthwashes, rapidly dissolving tablets, or lozenges. For transdermal administration, the active compounds are formulated into ointments, salves, gels, foams, or creams as generally known in the art.

[0071] The pharmaceutical compositions can be delivered using drug delivery systems. Such delivery systems include hyaluronic acid solutions or suspensions of collagen fragments. The drugs can be formulated in microcapsules, designed with appropriate
polymeric materials for controlled release, such as polylactic acid, ethylhydroxycellulose, polycaprolactone, polycaprolactone diol, polylysine, polyglycolic, polymaleic acid, poly[N-(2-hydroxypropyl)methylacrylamide] and the like. Particular formulations using drug delivery systems can be in the form of liquid suspensions, ointments, complexes to a bandage, collagen shield or the like.

The composition can further comprise any other suitable components, especially for enhancing the stability of the composition and/or its end-use. Accordingly, there is a wide variety of suitable formulations of the composition of the invention.

Sustained release compositions can also be employed in the present compositions, such as those described in, for example, U.S. Pat. Nos. 5,672,659 and 5,595,760. The use of immediate or sustained release compositions depends on the nature of the condition being treated. If the condition consists of an acute or over-acute disorder, treatment with an immediate release form will be preferred over a prolonged release composition. Alternatively, for certain preventative or long-term treatments, a sustained release composition may be appropriate.

In addition, the composition can comprise additional therapeutic or biologically-active agents. For example, therapeutic factors useful in the treatment of a particular indication can be present. Factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with in vivo administration of the pharmaceutical composition and physiological distress.

Compositions provided by the invention can include, e.g., from about 0.5 ml to about 4 ml aqueous or organic liquids with an active agent coupled to a SPARC binding antibody, with the concentration of the active agent from about 10 mg/ml to about 100 mg/ml, preferably from about 1 mg/ml to about 10 mg/ml, more preferably from about 0.1 mg/ml to about 1 mg/ml. The active agent can be present at any suitable and therapeutically effective concentration, e.g., Avastin at a concentration of from about 10 mg/ml to about 50 mg/ml.

Methods

The invention provides a method for diagnosing or treating a disease in an animal by administering a diagnostically or therapeutically effective amount of a composition comprising a SPARC binding antibody comprising Imm-2, Imm-3, or combinations thereof. In some embodiments, the invention provides a method for diagnosing a disease in an animal by administering an effective amount of Imm-2, Imm-3, or a combination thereof. In other
embodiments, the invention provides a method for treating a disease in an animal by
administering an effective amount of Imm-2, Imm-3, or a combination thereof. Any
composition described above can be used in the methods of the present invention.

According to the methods of the present invention, a therapeutically effective
amount of the composition can be administered to the mammal to enhance delivery of the
active agent to a disease site relative to delivery of the active agent alone, or to enhance
clearance resulting in a decrease in blood level of SPARC. In preferred embodiments, the
decrease in blood level of SPARC is at least about 10%. In more preferred embodiments, the
decrease in blood level of SPARC is at least about 15%, 20%, 25%, 30%, 35%, 40%, 45%,
or, most preferably, at least about 50%.

The invention also provides a method of diagnosing a disease or condition in an
animal comprising (a) administering to the animal a diagnostically effective amount of a
SPARC binding antibody comprising Imm-2, Imm-3, or a combination thereof; (b) detecting
the amount of SPARC binding antibody present in a particular site or tissue of the animal;
and (c) diagnosing that the disease or condition is present if the amount of SPARC binding
antibody present indicates that significantly greater than normal levels of SPARC are present
in the particular site or tissue.

The present methods can be used in any condition characterized by
overexpression of SPARC. Exemplary diseases for which the present invention is useful
include abnormal conditions of proliferation, tissue remodeling, hyperplasia, exaggerated
wound healing in any bodily tissue including soft tissue, connective tissue, bone, solid
organs, blood vessel and the like. Examples of diseases treatable or diagnosed using the
methods and compositions of the present invention include cancer, diabetic or other
retinopathy, inflammation, arthritis, restenosis in blood vessels or artificial blood vessel grafts
or intravascular devices and the like.

Other diseases within the scope of the methods of the present invention include,
without limitation, cancer, restenosis or other proliferative diseases, fibrosis, osteoporosis or
exaggerated wound healing. Specifically, such suitable diseases include, without limitation,
wherein: (a) the cancer can be, for example, circinoma in situ, atypical hyperplasia,
carcinoma, sarcoma, carcinosarcoma, lung cancer, pancreatic cancer, skin cancer,
haematological neoplasms, breast cancer, brain cancer, colon cancer, bladder cancer, cervical
cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancer, multiple
myeloma, liver cancer, leukemia, lymphoma, oral cancer, osteosarcomas, ovarian cancer,
prostate cancer, testicular cancer, and thyroid cancer, (b) the restenosis can be, for example, coronary artery restenosis, cerebral artery restenosis, carotid artery restenosis, renal artery restenosis, femoral artery restenosis, peripheral artery restenosis or combinations thereof, (c) the other proliferative disease can be, for example, hyperlasias, endometriosis, hypertrophic scars and keloids, proliferative diabetic retinopathy, glomerulonephritis, proliferative, pulmonary hypertension, rheumatoid arthritis, arteriovenous malformations, atherosclerotic plaques, coronary artery disease, delayed wound healing, hemophilic joints, nonunion fractures, Osier-Weber syndrome, psoriasis, pyogenic granuloma, scleroderma, tracoma, menorrhagia, vascular adhesions, and papillomas, and (d) the fibrotic disease can be, for example, hepatic fibrosis, pulmonary fibrosis and retroperitoneal fibrosis.

The animal can be any patient or subject in need of treatment or diagnosis. In preferred embodiments, the animal is a mammal. In particularly preferred embodiments, the animal is a human. In other embodiments, the animal can be a mouse, rat, rabbit, cat, dog, pig, sheep, horse, cow, or a non-human primate.

The invention also provides a method for destruction of SPARC expression tissues such as tumor and restenotic tissues via the complement fixation and/or recruitment of cell mediated immune response by a SPARC binding antibody.

The invention also provides a method for inhibition of SPARC activity using neutralizing antibody against SPARC, e.g., a suitable anti-SPARC antibody. A neutralizing antibody has the ability to block the interaction of SPARC with its effectors in vivo, for example, the interaction of SPARC with cell surface component or the binding of SPARC to its natural ligands such as albumin, growth factors, and Ca2+. The invention provides a method for delivering a chemotherapeutic agent to a tumor in a mammal. The methods comprise administering to a human or other animal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises the chemotherapeutic agent coupled to a suitable SPARC binding antibody and a pharmaceutically acceptable carrier. Descriptions of the chemotherapeutic agents, animals, and components thereof, set forth herein in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of delivering a chemotherapeutic agent to a tumor.

The types of tumor to be detected, whose response to chemotherapy can be predicted or determined, which can be treated in accordance with the invention are generally those found in humans and other mammals. The tumors can be the result of inoculation as
well, such as in laboratory animals. Many types and forms of tumors are encountered in human and other animal conditions, and there is no intention to limit the application of the methods of the present to any particular tumor type or variety. Tumors, as is known, include an abnormal mass of tissue that results from uncontrolled and progressive cell division, and is also typically known as a "neoplasm." The inventive methods are useful for tumor cells and associated stromal cells, solid tumors and tumors associated with soft tissue, such as, soft tissue sarcoma, for example, in a human.

The tumor or cancer can be located in the oral cavity and pharynx, the digestive system, the respiratory system, bones and joints (e.g., bony metastases), soft tissue, the skin (e.g., melanoma), breast, the genital system, the urinary system, the eye and orbit, the brain and central nervous system (e.g., glioma), or the endocrine system (e.g., thyroid) and is not necessarily limited to the primary tumor or cancer. Tissues associated with the oral cavity include, but are not limited to, the tongue and tissues of the mouth. Cancer can arise in tissues of the digestive system including, for example, the esophagus, stomach, small intestine, colon, rectum, anus, liver, gall bladder, and pancreas. Cancers of the respiratory system can affect the larynx, lung, and bronchus and include, for example, small cell and non-small cell lung carcinoma. Tumors can arise in the uterine cervix, uterine corpus, ovary, vulva, vagina, prostate, testis, and penis, which make up the male and female genital systems, and the urinary bladder, kidney, renal pelvis, and ureter, which comprise the urinary system. The tumor or cancer can be located in the head and/or neck (e.g., laryngeal cancer and parathyroid cancer). The tumor or cancer also can be located in the hematopoietic system or lymphoid system, and include, for example, lymphoma (e.g., Hodgkin's disease and Non-Hodgkin's lymphoma), multiple myeloma, or leukemia (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, and the like). Preferably, the tumor is located in the bladder, liver, ovary, kidney, gut, brain, or breast.

In other embodiments, the invention provides a method for delivering a pharmaceutically active agent by way of a SPARC binding antibody to a site of disease that is characterized by overexpression of SPARC. Such diseases include abnormal conditions of proliferation, tissue remodeling, hyperplasia, and exaggerated wound healing in bodily tissue (e.g., soft tissue, connective tissue, bone, solid organs, blood vessel and the like). Examples of diseases that are treatable or can be diagnosed by administering a pharmaceutical composition comprising a therapeutic agent coupled to a suitable SPARC antibody, include...
cancer, diabetic or other retinopathy, inflammation, arthritis, restenosis in blood vessels, artificial blood vessel grafts, or intravascular devices, and the like. Descriptions of the chemotherapeutic agents, tumors, animals, and components thereof, set forth herein in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of delivering a pharmaceutically active agent.

In other embodiments, the inventive methods comprise administering to a mammal a therapeutically effective amount of a pharmaceutical composition comprising a liposome bound or albumin bound chemotherapeutic agent wherein the liposome or albumin is coupled to a suitable disease targeting SPARC binding antibody. The chemotherapeutic agent can be coupled to the SPARC binding antibody using any suitable method. Preferably, the chemotherapeutic agent is chemically coupled to the compound via covalent bonds including, for example, disulfide bonds.

One or more doses of one or more chemotherapeutic agents, such as those described above, can also be administered according to the inventive methods. The type and number of chemotherapeutic agents used in the inventive method will depend on the standard chemotherapeutic regimen for a particular tumor type. In other words, while a particular cancer can be treated routinely with a single chemotherapeutic agent, another can be treated routinely with a combination of chemotherapeutic agents. Methods for coupling or conjugation of suitable therapeutics, chemotherapeutics, radionuclides, etc. to antibodies or fragments thereof are well described in the art. The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

Methods in accordance with the invention include, e.g., combination therapies wherein the animal is also undergoing one or more cancer therapies selected from the group consisting of surgery, chemotherapy, radiotherapy, thermotherapy, immunotherapy, hormone therapy and laser therapy. The terms "co-administration" and "combination therapy" refer to administering to a subject two or more therapeutically active agents. The agents can be contained in a single pharmaceutical composition and be administered at the same time, or the agents can be contained in separate formulation and administered serially to a subject. So long as the two agents can be detected in the subject at the same time, the two agents are said to be co-administered.

Combination therapies contemplated in the present invention include, but are not limited to antibody administration, vaccine administration, administration of cytotoxic agents, natural amino acid polypeptides, nucleic acids, nucleotide analogues, and biologic response
modifiers. Two or more combined compounds may be used together or sequentially. Examples of chemotherapeutic agents include alkylating agents, antimetabolites, natural products, hormones and antagonists, and miscellaneous agents. Examples of alkylating agents include nitrogen mustards such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan (L-sarcolysin) and chlorambucil; ethylenimines and methylmelamines such as hexamethylmelamine and thiopeta; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine (BCNU), semustine (methyl-CCNU), lomustine (CCNU) and streptozocin (streptozotocin); DNA synthesis antagonists such as estramustine phosphate; and triazines such as dacarbazine (DTIC, dimethyl-triazenomidazolecarboxamide) and temozolomide. Examples of antimetabolites include folic acid analogs such as methotrexate (amethopterin); pyrimidine analogs such as fluorouracil (5-fluorouracil, 5-FU, 5FU), floxuridine (fluorodeoxyuridine, FUDR), cytarabine (cytosine arabinoside) and gemcitabine; purine analogs such as mercaptopurine (6-mercaptopurine, 6-MP), thioguanine (6-thioguanine, TG) and pentostatin (2'-deoxycoformycin, deoxycoformycin), cladribine and fludarabine; and topoisomerase inhibitors such as amsacrine. Examples of natural products include vinca alkaloids such as vinblastine (VLB) and vincristine; taxanes such as paclitaxel (Abraxane) and docetaxel (Taxotere); epipodophyllotoxins such as etoposide and teniposide; camptothecins such as topotecan and irinotecan; antibiotics such as dactinomycin (actinomycin D), daunorubicin (daunomycin, rubidomycin), doxorubicin, bleomycin, mitomycin (mitomycin C), idarubicin, epirubicin; enzymes such as L-asparaginase; and biological response modifiers such as interferon alpha and interleukin 2. Examples of hormones and antagonists include luteinising releasing hormone agonists such as buserelin; adrenocortico steroids such as prednisone and related preparations; progestins such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogens such as diethylstilbestrol and ethinyl estradiol and related preparations; estrogen antagonists such as tamoxifen and anastrozole; androgens such as testosterone propionate and fluoxymesterone and related preparations; androgen antagonists such as flutamide and bicalutamide; and gonadotropin- releasing hormone analogs such as leuprolide. Examples of miscellaneous agents include thalidomide; platinum coordination complexes such as cisplatin (czs-DDP), oxaliplatin and carboplatin; anthracyclines such as mitoxantrone; substituted ureas such as hydroxyurea; methylhydrazine derivatives such as procarbazine (N-methylhydrazine, MIH); adrenocortical suppressants such as mitotane (o,ρ'-DDD) and
aminoglutethimide; RXR agonists such as bexarotene; and tyrosine kinase inhibitors such as imatinib.

Compositions featured in the methods of the present invention can be administered in a single dose or in multiple doses. Where the administration of the antibodies by infusion, the infusion can be a single sustained dose or can be delivered by multiple infusions. Injection of the agent can be directly into the tissue at or near the site of aberrant target gene expression. Multiple injections of the agent can be made into the tissue at or near the site.

Dosage levels on the order of about 1 ug/kg to 100 mg/kg of body weight per administration are useful in the treatment of a disease. In regard to dosage, an antibody can be administered at a unit dose less than about 75 mg per kg of body weight, or less than about 70, 60, 50, 40, 30, 20, 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, or 0.0005 mg per kg of body weight, and less than 200 nmol of antibody per kg of body weight, or less than 1500, 750, 300, 150, 75, 15, 7.5, 1.5, 0.75, 0.15, 0.075, 0.015, 0.0075, 0.0015, 0.00075, 0.00015 nmol of antibody per kg of body weight. The unit dose, for example, can be administered by injection (e.g., intravenous or intramuscular, intrathecally, or directly into an organ), inhalation, or a topical application.

One skilled in the art can also readily determine an appropriate dosage regimen for administering the antibody of the invention to a given subject. For example, the SPARC-binding antibody composition can be administered to the subject once, as a single injection or deposition at or near the site of SPARC expression. Compositions of the present invention can be administered daily, semi-weekly, weekly, bi-weekly, semi-monthly, monthly, bi-monthly, or at the discretion of the clinician. In some embodiments, the compositions are administered once or twice daily to a subject for a period of from about three to about twenty-eight days, more preferably from about seven to about ten days. In further embodiments, the unit dose is administered less frequently than once a day, e.g., less than every 2, 4, 8 or 30 days. In other embodiments, the unit dose is not administered with a frequency (e.g., not a regular frequency).

Where a dosage regimen comprises multiple administrations, it is understood that the effective amount of SPARC-binding antibody composition administered to the subject can include the total amount of antibody administered over the entire dosage regimen. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending on a variety of factors, including the specific SPARC binding antibody
composition being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the particular disorder being treated, the severity of the disorder, the pharmacodynamics of the oligonucleotide agent, and the age, sex, weight, and general health of the patient. Wide variations in the necessary dosage level are to be expected in view of the differing efficiencies of the various routes of administration.

The effective dose can be administered in a single dose or in two or more doses, as desired or considered appropriate under the specific circumstances. If desired to facilitate repeated or frequent infusions, implantation of a delivery device, e.g., a pump, semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular), or reservoir may be advisable. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state. The concentration of the antibody composition is an amount sufficient to be effective in treating or preventing a disorder or to regulate a physiological condition in humans. The concentration or amount of antibody administered will depend on the parameters determined for the agent and the method of administration.

Certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. It will also be appreciated that the effective dosage of the antibody used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays. For example, the subject can be monitored after administering an antibody composition. Based on information from the monitoring, an additional amount of the antibody composition can be administered. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

EXAMPLE 1

This Example demonstrates the preparation of a series of antibodies capable of binding to human SPARC.

Twelve mouse-derived anti-human SPARC antibodies were commercially generated using a conventional hybridoma approach using mouse strain RBF/DnJ.

A pASK84 expression vector (Figure 1) was used to express the Fab regions of the resulting antibodies, designated Imml-Imm-12. The Fab regions were targeted to the
periplasm where they were collected and subsequently purified via activity chromatography on a protein A sepharose column. Identity was verified by Western blot and SPARC binding activity was verified by ELISA.

[00101] Imm-13 and 14 are fully human anti-human SPARC antibodies which were generated using a human phage display library. SPARC was panned against the commercial human Fab phage display library HuFabL® (Creative Biolabs, Shirley, NY). Two Fab sequences of interest were identified: Fab6 (SEQ ID NO 15) and Fab16 (SEQ ID NO 16), as shown in Figure 2. SPARC binding activity was verified by ELISA for these two Fab molecules.

[00102] These Fab regions were cloned into the pBAD vector (Figure 3) and were expressed and purified in bacteria. The Fab proteins expressed by the pBAD vector were isolated from the periplasmic fraction of lysed bacteria, with sequences provided at Figure 4. The identities of the Fab regions obtained from the periplasmic fraction were verified by SDS page. The Fab proteins were purified to homogeneity via activity chromatography on a protein A sepharose column.

[00103] In order to create fully human anti-SPARC antibodies, the genes for Fab6 and Fab16 were cloned and expressed via the pcDNA3002Neo Vector (Invitrogen, Carlsbad, CA) (Figure 21). The resultant antibodies were purified and their identities were verified by gel electrophoresis and N-terminal analysis. The fully human antibody created from Fab6 was designated Imm-13 and the fully human antibody created from Fab16 was designated Imm-14.

[00104] After they were generated according to the foregoing methods, Imm-1 through Imm14 antibodies were characterized according to isotype by utilizing a commercial mouse isotyping test kit (AbD Serotec, Raleigh, NC). The results are presented in Table 1.

<table>
<thead>
<tr>
<th>Clone Number</th>
<th>Abraxis Name</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Imm-1</td>
<td>IgGl (K)</td>
</tr>
<tr>
<td>38</td>
<td>Imm-2</td>
<td>IgGl, 2b (K)</td>
</tr>
<tr>
<td>39</td>
<td>Imm-3</td>
<td>IgGl, 2b (K)</td>
</tr>
<tr>
<td>43</td>
<td>Imm-4</td>
<td>IgGl (K)</td>
</tr>
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</tr>
<tr>
<td>47</td>
<td>Imm-5</td>
<td>IgG2a (K)</td>
</tr>
<tr>
<td>49</td>
<td>Imm-6</td>
<td>IgGl (K)</td>
</tr>
<tr>
<td>55</td>
<td>Imm-7</td>
<td>IgG2a (K)</td>
</tr>
<tr>
<td>58</td>
<td>Imm-8</td>
<td>IgG2b (K)</td>
</tr>
<tr>
<td>62</td>
<td>Imm-9</td>
<td>IgGl (K)</td>
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<tr>
<td>70</td>
<td>Imm-10</td>
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<tr>
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<td>IgGl (K)</td>
</tr>
<tr>
<td>F16</td>
<td>Imm-14</td>
<td>IgGl (K)</td>
</tr>
</tbody>
</table>

[00105] The sequences for the variable complimentary determining regions for selected Imm-series antibodies, including Imm-2 and Imm-3, are presented in Figure 5. The clones in Table 1, Imm-1 through Imm-14, will be deposited at a suitable depository, such as the ATTC.

EXAMPLE 2

[00106] This Example demonstrates the use of ELISA assays to characterize the SPARC binding of the Imm-series antibodies.

[00107] The ability of Imm-1 through Imm-12 (the mouse-derived anti-human SPARC antibodies) to bind recombinant human SPARC (Biol-SPARC) was characterized by multiple ELISA assays performed at various stages of purification. Figure 6 presents the results of an ELISA assay performed on a serial dilution (1:1, 1:10, and 1:100) of antibody supernatants prior to purification. In this assay, Imm-4, Imm-6, Imm-9, Imm-10 and Imm-12 exhibited the highest Biol-SPARC binding, with Imm-12 exhibiting the highest binding overall. Another ELISA assay was performed with the purified antibodies (Figure 7) at concentrations of 0.04 µg/mL, 0.2 µg/mL, 1 µg/mL, and 5 µg/mL. The binding of the purified antibodies was generally improved over the unpurified supernatants. In this assay, Imm-4, Imm-9, Imm-11 and Imm-12 exhibited the highest Biol-SPARC binding. An additional ELISA was performed to compare the binding of the mouse derived Imm-series
antibodies to two different varieties of SPARC: Biol-SPARC, and human platelet SPARC (HTI-SPARC) (Figure 8). In this assay, Imm-4 and Imm-9 were both found to bind Biol-SPARC significantly better than HTI-SPARC. Imm-11 and Imm-12 bind both varieties of human SPARC equally well.

[0100] ELISA assays were also used to characterize the SPARC binding of the fully human anti-SPARC antibodies, Imm-13 and Imm-14. For example, according to a protein ELISA assay (Figure 9), Fab16 (the Fab region of Imm-14) binds HTI-SPARC with a $K_D$ of 11 nM and binds Biol-SPARC with a $K_D$ of 7 nM. Surface plasmon resonance binding assays, performed on the Biacore 3000® (GE/Biacore International AB, Uppsala, Sweden), tested the binding of Fab16 to both varieties of SPARC immobilized on a sensorchip (Figures 10 and 11). These assays resulted in $K_D$ values of 76.2 nM for HTI SPARC and 132 nM for Biol-SPARC.

[0101] An ELISA assay was also performed to directly compare the SPARC binding capabilities of selected mouse-derived anti human SPARC antibodies, Imm-11 and Imm-12, to the fully human Imm-13 and Imm-14, the results of which are presented in Figure 24. The results indicate that Imm-13 has a higher affinity for SPARC than both of the mouse derived antibodies, while Imm-14 has a lower affinity.

[0102] This example demonstrates that certain of the Imm series antibodies bind, in vitro, to both recombinant human SPARC and human platelet SPARC in binding assays.

EXAMPLE 3

[0103] This example demonstrates the analysis of the epitopes to which the Imm-series antibodies bind.

[0104] Western blotting was used to determine whether the Imm-series antibodies bind to linear or conformational epitopes. In this analysis, SPARC protein was run on a polyacrylamide gel in the presence of SDS. Accordingly, the SPARC protein on the gel was in its denatured form. The Imm-series antibodies were used as primary antibodies and were then probed with goat anti-mouse IgG. BSA was used as a negative control. The results of the assay, shown at Figure 13, show binding of Imm-11 and Imm-12 to SPARC. Binding to the other Imm series antibodies was not detected by this assay.

[0105] Further epitope mapping analyses using phage display. In brief, the mAbs were exposed to phage display library, washed to remove nonspecific binders, and the bound phages eluted and sequenced. The peptide sequences provided at Figures 15-16 confirm that
several of the Imm-series antibodies were found to recognize different epitopes with some spanning multiple sequence positions when the phage sequences were aligned against SPARC protein sequence using Clone Manager program (Figure 14).

These results show that Imm-1 1 and Imm-12 bind SPARC based on linear, or primary, epitopes, while the remaining Imm-series antibodies bind SPARC at epitopes of higher order structure rather than on the primary amino acid sequence alone.

EXAMPLE 4

This example demonstrates the ability of Imm-2 and Imm-3 to localize at a tumor site in an in vivo tumor model.

Nude mice implanted with subcutaneous HT29 colon xenografts were treated with Imm series antibodies labeled with labeled with Alexa 680 fluorescent dye at dose of 200 ug/mouse. The labeled Imm antibodies were formulated in saline and administered intravenously on day 1. The fluorescent signal was followed in these mice over the course of 36 days.

Figure 17 depicts the results of this study. Imm-2 and Imm-3 show greater tumor localization than other evaluated antibodies. Tumor specific localization was was detectable as early as one day after initial administration and progressively increased until day 36. Figures 18 and 19 provide exemplary display of Imm-2’s ability to visualize tumors at days 18 and 28 respectively.

Despite strong SPARC binding in ELISA assays, Imm-4, Imm-9, Imm-11, Imm-12 and Imm-13 were not able to localize to the tumor in vivo.

These data indicate that SPARC epitopes are generally not available in vivo. Accordingly Imm-2 and Imm-3, which are able to localize to the tumor in vivo, are likely to be uniquely useful in the treatment, diagnosis and real-time imaging of tumors in patients.

EXAMPLE 5

This Example discusses results of in vivo assay examining the effect of certain Imm-series antibodies on survival in nude mice challenged with LL/2 Lewis Lung Carcinoma. The animals in the study were administered either Imm-2, Imm-12, or Imm-14. A negative control group was administered mlgG. The antibodies were formulated in PBS and were administered in at dose of 200 ug/mouse, 2 x wkly, for four weeks. The survival of the animals was then recorded over twenty days.
A higher percentage of animals treated with Imm-2 survived at the various time points than did animals treated with Imm-12 or Imm-14. Figure 20 depicts the results of the study.

These results indicate that Imm-2 may be useful in treating cancer.

EXAMPLE 6

This example illustrates the use of the antibodies of the present invention to diagnose a proliferative disease.

A suitable quantity of Imm-2 and/or Imm-3 is prepared in accordance with the methods described above or via other methods known in the art. The antibodies are conjugated to a diagnostic agent suitable for medical imaging, such as a radionuclide, using a conjugation method known in the art.

The composition is applied to tissue samples taken from a test cohort of patients suffering from a proliferative disease associated with the overexpression of SPARC, e.g., breast cancer. The composition is likewise applied to tissue samples taken from a negative control cohort, not suffering from a proliferative disease.

The use of appropriate medical imaging techniques on the test cohort samples indicates the presence of disease, while the same techniques applied to the control cohort samples indicate the absence of disease.

The results will show that the antibodies of the present invention are useful in diagnosing proliferative diseases.

EXAMPLE 7

This example illustrates the use of the antibodies of the present invention to treat a proliferative disease in a mouse tumor model.

A suitable quantity of Imm-2 and/or Imm-3 is prepared in accordance with the methods described above or via other methods known in the art. The antibodies are conjugated to a chemotherapeutic agent, such as Doxil, using an appropriate conjugation method known in the art. The conjugate is formulated in an aqueous composition.

The composition is administered intravenously, in one or more doses, to a test cohort of mice suffering from a proliferative disease associated with the overexpression of SPARC, e.g., a breast cancer model. A control cohort, not suffering from a proliferative
disease is administered the identical composition intravenously, according to a corresponding dosage regimen.

[0123] Pathological analysis of tumor samples and/or mouse survival indicate that mortality and/or morbidity are improved in the test cohort over the control cohort.

[0124] The results will show that the antibodies of the present invention are useful in treating proliferative diseases.

[0125] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0126] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to," unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0127] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible
variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.
CLAIM(S):

1. A composition comprising a SPARC binding antibody, wherein the SPARC binding antibody comprises Imm-2, Imm-3, or a combination thereof.

2. The composition of claim 1 wherein the SPARC binding antibody comprises Imm-2.

3. The composition of claim 1 wherein the SPARC binding antibody comprises Imm-3.

4. The composition of claim 1 wherein the SPARC binding antibody comprises both Imm-2 and Imm-3.

5. The composition of any of claims 1 through 4, further comprising an active agent, wherein the active agent is conjugated to the SPARC binding antibody.

6. The composition of claim 5, wherein the active agent comprises a therapeutic agent or a diagnostic agent.

7. The composition of claim 6, wherein the therapeutic agent or diagnostic agent is a therapeutic agent selected from the group consisting of tyrosine kinase inhibitors, kinase inhibitors, biologically active agents, biological molecules, radionuclides, adriamycin, ansamycin antibiotics, asparaginase, bleomycin, busulphan, cisplatin, carboplatin, carmustine, capecitabine, chlorambucil, cytarabine, cyclophosphamide, camptothecin, dacarbazine, daclomycin, daunorubicin, dexrazoxane, docetaxel, doxorubicin, etoposide, epothilones, floxuridine, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idamycin, ifosfamide, irinotecan, lomustine, mechlorethamine, mercaptopurine, mephalan, methotrexate, rapamycin (sirolimus), mitomycin, mitotane, mitoxantrone, nitrosourea, paclitaxel, pamidronate, pentostatin, plicamycin, procarbazine, rituximab, streptozocin, teniposide, thioguanine, thiopeta, taxanes, vinblastine, vincristine, vinorelbine, taxol, combretastatins, discodermolides, transplatinum, anti-vascular endothelial growth factor compounds ("anti-VEGFs"), anti-epidermal growth factor receptor compounds ("anti-EGFRs"), 5-fluorouracil and derivatives, radionuclides, polypeptide toxins, apoptosis inducers, therapy sensitizers, enzyme or active fragment thereof, and combinations thereof.

8. The composition of claim 6, wherein the therapeutic agent or diagnostic agent is a therapeutic agent comprising an antibody or antibody fragment.

9. The composition of claim 8, wherein said antibody or antibody fragment is a Fc fragment of IgG, or IgA, or IgD, or IgE, or IgM.
10. The composition of claims 8 or 9, wherein said antibody or antibody fragment mediates one or more of complement activation, cell mediated cytotoxicity or opsonization, or mast cell activation, or other immune response.

11. The composition of claim 6, wherein the therapeutic agent or diagnostic agent is a diagnostic agent selected from the group consisting of fluorochromes, radioactive agents, MRI contrast agents, X-ray contrast agents, ultrasound contrast agents, and PET contrast agents.

12. The composition of any one of claims 1-11, wherein the composition is contained in a liposome.

13. The composition of any one of claims 1-11, wherein the composition is contained in an albumin nanoparticle.

14. The composition of any of claims 1-13, wherein the composition further comprises a suitable pharmaceutical carrier.

15. The composition of any of claims 1-16, wherein said composition is administered to a patient via i.v., topically, via injection, via inhalation, intranasally, rectally or orally.

16. A method for diagnosing or treating a disease in an animal comprising: administering a diagnostically or therapeutically effective amount of a composition comprising a SPARC binding antibody, wherein the SPARC binding antibody comprises Imm-2, Imm-3, or a combination thereof.

17. The method of claim 16 wherein the SPARC binding antibody comprises Imm-2.

18. The method of claim 16 wherein the SPARC binding antibody comprises Imm-3.

19. The method of claim 16 wherein the SPARC binding antibody comprises both Imm-2 and Imm-3.

20. The method of any of claims 18-22, wherein the composition further comprises an active agent conjugated to the SPARC binding antibody.

21. The method of claim 20, wherein the active agent comprises a therapeutic agent or a diagnostic agent.

22. The method of claim 21, wherein the therapeutic agent or diagnostic agent is a therapeutic agent selected from the group consisting of tyrosine kinase inhibitors, kinase inhibitors, biologically active agents, biological molecules, radionuclides, adriamycin,
ansamycin antibiotics, asparaginase, bleomycin, busulphan, cisplatin, carboplatin, carmustine, capecitabine, chlorambucil, cytarabine, cyclophosphamide, camptothecin, dacarbazine, dactinomycin, daunorubicin, dexrazoxane, docetaxel, doxorubicin, etoposide, epothilones, floxuridine, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine, mechlorethamine, mercaptopurine, mephalalan, methotrexate, rapamycin (sirolimus), mitomycin, mitotane, mitoxantrone, nitrosurea, paclitaxel, pamidronate, pentostatin, plicamycin, procarbazine, rituximab, streptozocin, teniposide, thioguanine, thiotretoin, taxanes, vinblastine, vincristine, vinorelbine, taxol, combretastatins, discodermolides, transplatinum, anti-vascular endothelial growth factor compounds ("anti-VEGFs"), anti-epidermal growth factor receptor compounds ("anti-EGFRs"), 5-fluorouracil and derivatives, radionuclides, polypeptide toxins, apoptosis inducers, therapy sensitizers, enzyme or active fragment thereof, and combinations thereof.

23. The method of claim 21, wherein the therapeutic agent or diagnostic agent is a therapeutic agent comprising an antibody or antibody fragment.

24. The method of claim 23, wherein the antibody or antibody fragment is a Fc fragment of IgG, or IgA, or IgD, or IgE, or IgM.

25. The method of claims 23 or 24, wherein said antibody or antibody fragment mediates one or more of complement activation, cell mediated cytotoxicity or opsonization, or mast cell activation, or other immune response.

26. The method of claim 21, wherein the therapeutic agent or diagnostic agent is a diagnostic agent selected from the group consisting of fluorochromes, radioactive agents, MRI contrast agents, X-ray contrast agents, ultrasound contrast agents, and PET contrast agents.

27. The method of any one of claims 16-26, wherein the composition further comprises a suitable pharmaceutical carrier.

28. The method of any one of claims 16-27, wherein the therapeutically effective amount of the composition is administered to a patient via i.v., topically, via injection, via inhalation, intranasally, rectally or orally.

29. The method of any one of claims 16-28, further comprising administering a therapeutically effect amount of albumin bound nanoparticulate paclitaxel.

30. The method of claim 16, wherein the tumor is selected from the group consisting of oral cavity tumors, pharyngeal tumors, digestive system tumors, respiratory system tumors, bone tumors, cartilaginous tumors, bone metastases, sarcomas, skin tumors, melanoma, breast tumors, genital system tumors, urinary tract tumors, orbital tumors, brain

31. The method of any one of claims 16-30, wherein the animal is human.
Fab16 in pBAD

SEQ ID NO 17

VL-CL:
MKKTAIAIAVALAGFATTQAELVLTQPSSVSTPGQRVTTSCSGSSSNIGSNYVYYQQLPGTAPKLLIYRN
NQRPSGVDPDRFSGSKSGTSSALIASGRLSEDEADYYCAAWDDSLSGWVFGGKTLTVLQPKAAPSVTLFPPSS
EELQANKATLVCLISDFYPGAVTVWAKADGSPVKAGVETTTTPSKQSNKNYAASSYLSLTPEQWKSHEHRSYSQVT
HEGSTVEKTVAPTECS

VH-CH1
MKYLLPAAAAGLLLLAAQPAMAQVLQVSQGGGLIQPGSSLRLSCAASGFTVSSNYMWSWRQAPGKLEWVSYIY
SGGSTYYADSVKGRFTISRDNSKNTLYQMNSLAEDETAVVYCARIGIAAAGLDYWGQGTLVTVSASTKGFPSVF
PLAPSSKSTSGTAALGCLVKDYFPEPVTVSNAGTSGVHTFPAVLQSSGLYSSVSVTVPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTSQAGQHHHHHGAYPYDVPDYAS
Figure 8

1. Imm1, 3, 4, 7, 9, and 10 show stronger binding to Bio1.

2. Imm11 and 12 are strong binders to both SPARC types.
Figure 10

Fab16 on HTI SPARC

Fig. 16 = SEQ ID NO 20
<table>
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<tr>
<th>Imm13</th>
<th>imm14</th>
<th>Imm11</th>
<th>Imm12</th>
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<tbody>
<tr>
<td>One site binding (hyperbola)</td>
<td>3.259</td>
<td>3.296</td>
<td>3.164</td>
</tr>
<tr>
<td>Emax (Absorbance Signal)</td>
<td>7454</td>
<td>9469</td>
<td>1920</td>
</tr>
<tr>
<td>Kd (ng/mL)</td>
<td>0.05</td>
<td>0.63</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**Figure 12**

[Graph with data points and axes labels]
Figure 14

Epitope Mappings of anti-SPARC Antibodies
Peptide Sequences from Phage Display Library against Imm-series Aligned against SPARC

<table>
<thead>
<tr>
<th>mAb</th>
<th>pAb</th>
<th>Imm 1</th>
<th>Imm 2</th>
<th>Imm 3</th>
<th>Imm 4</th>
<th>Imm 7</th>
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</thead>
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<tr>
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<td>DQNLFGTPYHDFND</td>
<td>HRTSTVYSHITL</td>
<td>WIFLLKHANTPD</td>
<td>KSHKATXTRAS</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#2</td>
<td>DRQLQSYRTAL</td>
<td>YEDPSLSLPTFND</td>
<td>IDRKEFYEDNL</td>
<td>LKYRTSYDFHDF</td>
<td>DSNWVMTNTTYS</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#3</td>
<td>TSGPMPQAMNP</td>
<td>ACTMLSSLRWWD</td>
<td>ELSDVQYFRRFS</td>
<td>APTFQTVAMT</td>
<td>DSNWVMTNTTYS</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#4</td>
<td>GRQYQSYSTPD</td>
<td>NCQTQKKNWWK</td>
<td>ELRSKRSYVRK</td>
<td>GWAWSNHQVFL</td>
<td>EKTSQVQKFFYS</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#5</td>
<td>GQPTAISTPAQP</td>
<td>YTRSNSSSHVTD</td>
<td>AEKQVSLKSH</td>
<td>WKKHPFSGQSN</td>
<td>GKKHELMEQHR</td>
<td>THYKRTLSTAPV</td>
</tr>
<tr>
<td>#6</td>
<td>TQGIPPOAMNP</td>
<td>AGQINYFQWWD</td>
<td>DLTPKANPMF</td>
<td>AEDLQVSPHEK</td>
<td>DSNWVMTNTTYS</td>
<td>HGRRLQKVQDP</td>
</tr>
<tr>
<td>#7</td>
<td>UVQGQSYSSIII</td>
<td>TPITLPSNAGG</td>
<td>ELSDVQYFRRFS</td>
<td>APTFQTVAMT</td>
<td>DSNWVMTNTTYS</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#8</td>
<td>GLQSNSTFELIM</td>
<td>SKEQSSTVOSYQ</td>
<td>HWSQUHPLPS</td>
<td>GNGNNSRSHJLQ</td>
<td>DSNWVMTNTTYS</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#9</td>
<td>NTATQSYSTRAI</td>
<td>TPFQHPVQAGF</td>
<td>MSHKVRPAALL</td>
<td>LHRQHSHFSEE</td>
<td>EKKWMLHHPL</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#10</td>
<td>WTSTISTVSTLA</td>
<td>TTMHRSEQWHD</td>
<td>GHSQTEGKFWQ</td>
<td>HGKQGKSHKQP</td>
<td>HPGKQGTQPMP</td>
<td>SPLTVPEKVL</td>
</tr>
<tr>
<td>#11</td>
<td>NVLVEHSTAPM</td>
<td>GPTAMLEKCSGD</td>
<td>GSSEWQHQPMP</td>
<td>APTFQTVAMT</td>
<td>FSHQDNPQNNR</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#12</td>
<td>VQMGQSTMYGCA</td>
<td>NEQNAEWTGWW</td>
<td>NVLSWQHQPMP</td>
<td>APTFQTVAMT</td>
<td>DSNWVMTNTTYS</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#13</td>
<td>LQSTAYAMLTH</td>
<td>DQLLHEAGNSD</td>
<td>AETKQVSLKSH</td>
<td>GHSKTEGKFWQ</td>
<td>AERFQKTEKHP</td>
<td>DSNWVMTNTTYS</td>
</tr>
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<td>#14</td>
<td>ATATPSYSTRAI</td>
<td>ITKNKAPKMSD</td>
<td>NEEASLPTSN</td>
<td>HRESKQYAFQ</td>
<td>LKFNEFMTWKRL</td>
<td>DSNWVMTNTTYS</td>
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<tr>
<td>#15</td>
<td>ELHAGHMYRAS</td>
<td>QNMTFTPQHWD</td>
<td>AETKQVSLKSH</td>
<td>GHSKTEGKFWQ</td>
<td>AERFRGTEKHP</td>
<td>DSNWVMTNTTYS</td>
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<tr>
<td>#16</td>
<td>THRLWKEKTR</td>
<td>TTWMHHTISWDC</td>
<td>FQPKHSHQRLS</td>
<td>GHSKTEGKFWQ</td>
<td>QHNQKQPPSTV</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#17</td>
<td>TEHMSLAVSST</td>
<td>LGQPSQVWHD</td>
<td>SWMQTSGQRHR</td>
<td>WQPKHSHQRLS</td>
<td>HGQYVSQKFLQ</td>
<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#18</td>
<td>VLTQASTYKAP</td>
<td>WQPSELTAEMD</td>
<td>ELSDVQYFFRFS</td>
<td>FSPTQQTPWLYA</td>
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<td>DSNWVMTNTTYS</td>
</tr>
<tr>
<td>#19</td>
<td>TVMSSSYKRTT</td>
<td>NQMTFTPQHWD</td>
<td>KESHFSETSMS</td>
<td>APTFQTVAMT</td>
<td>AETKQVSLKSH</td>
<td>YRPNASQALIP</td>
</tr>
<tr>
<td>#20</td>
<td>QGEHSSSTYRSPS</td>
<td>SMHTTWREIFE</td>
<td>WQPKHSHQRLS</td>
<td>WQPKHSHQRLS</td>
<td>QHNQKQPPSTV</td>
<td>DSNWVMTNTTYS</td>
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</tbody>
</table>
# Figure 16

## Peptide Sequences from Phage Display Library against Imm-series Aligned against SPARC

<table>
<thead>
<tr>
<th>Imm 9</th>
<th>Imm 10</th>
<th>Imm 11</th>
<th>Imm 12</th>
<th>Imm 13</th>
<th>Imm 14</th>
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<tbody>
<tr>
<td>HAGHSHSKSHQRA</td>
<td>DNMHETIVDRTE</td>
<td>HMPLIQQFYWD</td>
<td>MMQRAYHYLLSQ</td>
<td>LQNLGQVRKRD</td>
<td>FPPSNLAAASNGF</td>
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<tr>
<td>HAGHSHSKSHQRA</td>
<td>STSTSSKNNHRR</td>
<td>GSTSTNSVPVRD</td>
<td>WRPQNSRLTEP</td>
<td>AFDLMLDRLER</td>
<td>SYMQZTFLAYH</td>
</tr>
<tr>
<td>KILHARQWTRM</td>
<td>EIVLARKHFEQQ</td>
<td>KPRKMISNHAY</td>
<td>VEFLGYPFFRRA</td>
<td>AFDLMLDRLER</td>
<td>MCDPVQAQREJRH</td>
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<td>HAWHRFGTSYLQG</td>
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<td>QFRQPIITSHWT</td>
<td>AFDLMLDRLER</td>
<td>AVSTFPLAVDLF</td>
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<tr>
<td>VWMPSHERATKT</td>
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<tr>
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<td>ATANASSYATTP</td>
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<td></td>
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<tr>
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<td>TTVSFSLGQKHL</td>
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<td>VVLKFEKDVRTT</td>
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<td>QSYHDTQGRD</td>
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<tr>
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## A. Classification of Subject Matter

**USPC - 424/130.1**

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)

- **IPC(8)**: A61K 39/395 (2011.01)
- **USPC**: 424/130.1

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

**USPC**: 530/388.15 (Text Search)

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

- PubMed (PGPB), USPTO, USOC, EPAB, JFAB; Google Scholar; PubMed: SPARC, antibody, mAb, Inm-S, targeting, therapeut, diagnoS, imagiS, drug, tumor, cancer, neoplastS
- GenCore: 6.3: SEQ ID NO: 2-3 and 9-10

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## C. Documents Considered to Be Relevant

<table>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>US 2009/004192 A1 (PEDERSEN et al.) 01 January 2009 (01.01.2009) SEQ ID NO: 81</td>
<td>1-9, 11, 16-24, 26 and 30</td>
</tr>
</tbody>
</table>

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* Further documents are listed in the continuation of Box C.

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

---

**Date of the actual completion of the international search:** 30 June 2011 (30.06.2011)

**Date of mailing of the international search report:** 19 JUL 2011

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**PCT OSP:** 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

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

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

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

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

3. ☒ Claims Nos. 10, 12-15, 25, 27, 29 and 31
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

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