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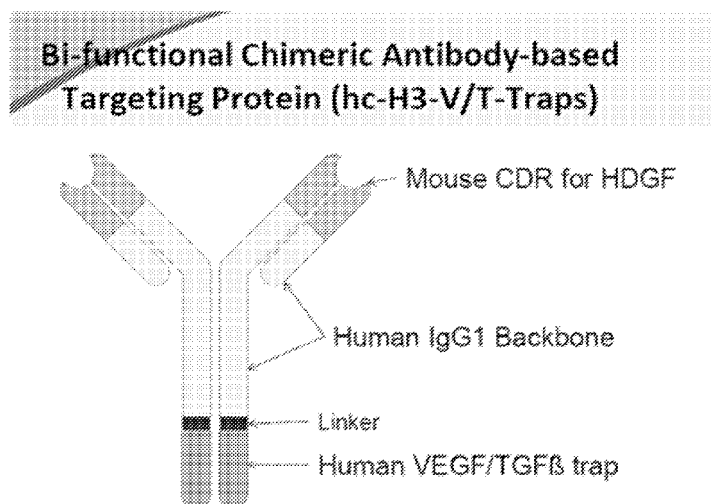


FIG. 1

(57) **Abstract:** This description provides novel strategies and methods for treatment of hyperproliferation conditions such as keloids, lung fibrosis, and lung cancer with the use of bi-functional molecules that target growth factors. A bifunctional hepatoma-derived growth factor (HDGF)-specific antibody for these methods comprises at least one complementarity determining region (CDR) specific for HDGF, at least one receptor domain that specifically binds to a growth factor selected from vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGFβ).



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METHODS OF TREATING CANCER USING BIFUNCTIONAL MOLECULES THAT TARGET GROWTH FACTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of Provisional Appln. 62/375,894, filed August 16, 2016, the entire contents of which are hereby incorporated by reference as if fully set forth herein, under 35 U.S.C. §119(e).

BACKGROUND

[0002] Lung diseases including lung cancer and pulmonary fibrosis (PF) are a leading cause of death worldwide. PF is one of a family of related diseases called interstitial lung diseases that can result in lung scarring. As the lung tissue becomes scarred, it interferes with a person's ability to breathe. Hyperproliferation of cells (e.g., fibroblasts or cancer cells) can cause various types of medical conditions (i.e., hyperproliferative conditions) such as keloids, idiopathic pulmonary fibrosis, and lung cancer. Keloids are a type of abnormally formed scar which is composed mainly of collagen as a result of an overgrowth of fibroblasts during wound healing. While keloid scars are benign and not contagious, it may be accompanied by severe itchiness, pain, and changes in skin texture. On the other hand, interstitial lung diseases, particularly idiopathic pulmonary fibrosis, are life threatening and have no cure currently.

[0003] Hepatoma-derived growth factor (HDGF) is a heparin-binding growth factor identified from media conditioned by a human hepatoma-derived cell line. It produces mitogenic activity in various cells types. Normally, HDGF is highly expressed during embryonic development in smooth muscle, gut, and endothelium, but not after birth. It has also been implicated in angiogenesis. High-level HDGF is observed in various human cancers. Specifically, HDGF is overexpressed in lung cancer and is a novel mitogenic growth factor for fibroblasts, vascular endothelial cells, and smooth muscle cells. High HDGF expression can also be found in keloid scar tissues but not in normal scar tissue. The molecular mechanisms of HDGF in cancer progression are poorly understood but we have demonstrated that lung cancer cells with down-regulated HDGF formed significantly smaller tumors in vivo (Zhang J., et al., "Down-regulation of hepatoma-derived growth factor inhibits anchorage-independent growth and invasion of non-small cell lung cancer cells," *Cancer Res* 2006; 66: 18-23). Diseases of the lung remain difficult to treat effectively. Therefore, a

novel strategy to prevent and treat diseases due to abnormal proliferation of cells (i.e., fibroblasts, cancer cells) is needed.

SUMMARY OF THE INVENTION

[0004] It has been discovered that it is possible to treat a hyperproliferative condition (e.g., keloids, interstitial lung disease, lung cancer) with a single bi-functional chimeric antibody which simultaneously targets HDGF and other over-produced angiogenic factors (i.e., VEGF and TGF- β) in tissue that is damaged, scarred, or cancerous. The simultaneous targeting of these growth factors using a single molecule allows for easier administration and improved efficacy.

[0005] Embodiments of the invention include a bifunctional hepatoma-derived growth factor (HDGF)-specific antibody (e.g., H3K, hH3K, H3T and hH3T) comprising at least one complementarity determining region (CDR) specific for HDGF and at least one receptor domain that also specifically binds to a growth factor selected from vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF β). The CDR may be selected from a mouse, human, rabbit, rat or other mammalian CDR, preferably a mouse CDR. The antibody may be humanized or chimeric and comprises two receptor domains each of which independently binds to a growth factor selected from vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF β) (i.e., a TGF β selected from TGF β subtype 1, subtype 2, and subtype 3). In certain aspects of the invention, the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody is linked to a reporter molecule selected from the group consisting of an enzyme, a radiolabel, a hapten, a fluorescent label, a phosphorescent molecule, a chemiluminescent molecule, a chromophore, a luminescent molecule, a photoaffinity molecule, a ligand, a colored particle or biotin. In the alternative, the antibody is linked to an effector molecule selected from the group consisting of, a toxin, and an apoptotic-inducing molecule, an antitumor agent, a therapeutic enzyme or a cytokine. In some aspects, the antitumor agent is selected from the group consisting of gemcitabine, pemetrexed, cisplatin, docetaxel, vinorelbine, doxorubicin, 6-fluorouracil, erlotinib, gefitinib, and crizotinib.

[0006] In some embodiments, pharmaceutical compositions comprising the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody with a pharmaceutically acceptable carrier and kits comprising them are provided. The pharmaceutical composition can be formulated for parenteral, intravenous or topical administration. Preferably the

pharmaceutical composition is formulated for administration in an amount from 5 to 25 mg/kg every 1-3 weeks.

[0007] Further embodiments include methods of reducing the growth of hyperproliferative cells in a subject by administering to the subject the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody in an amount of that down regulates HDGF and VEGF expression or HDGF and TGF β expression and is effective to reduce the abnormal growth of the hyperproliferative cells. In some embodiments, the hyperproliferative cell may be a fibroblast or a cancer cell. The cancer cell can be selected from the group consisting of lung, pancreas, colon, ovarian, liver, glioblastoma, and squamous cell carcinoma.

[0008] In other embodiments, methods are provided for simultaneously reducing expression of HDGF and VEGF or HDGF and TGF β in a subject in need thereof by administering a bifunctional HDGF-specific antibody or a combination of the bifunctional HDGF-specific antibodies (e.g., hH3K and hH3T). The bifunctional antibody can also be incorporated into a device, which may allow controlled release of the antibody to a needed site. In some embodiments, the device may be a scaffold, porous material, micro-encapsulating material or an infusion device (e.g., a coronary stent, or a wound dressing). The subject in need suffers from a hyperproliferative condition selected from the group consisting of interstitial lung disease, keloids, lung fibrosis, idiopathic pulmonary fibrosis, lung cancer, pancreatic cancer, colon cancer, ovarian cancer, liver cancer, glioblastoma, and squamous cell carcinoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The following drawings form part of the present specification and are included to further demonstrate certain embodiments of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0010] FIG. 1 is a schematic diagram illustrating a bi-functional chimeric antibody-based targeting protein (hc-H3-V-T-traps) specific embodiments of which are referred to as H3K or H3T.

[0011] FIG. 2A and FIG. 2B are images of western blots illustrating immunoprecipitation data for hc-anti HDGF H3 and hc-H3-V/T-traps.

[0012] FIG. 3 is a graph illustrating growth curves of PDX model 2131-8 receiving different treatments and showing that anti-HDGF antibody inhibits tumor drug resistance. Human Patient Derived tumor Xenograph (PDX) model 2131-8, a poorly differentiated adenocarcinoma of the lung, was implanted on the flank of athymic nude mice. Established

tumors (150-400 mm³, 10 tumors per group) were treated with chemotherapy or combined chemotherapy plus antibody regimen. Arm A (top curve): PBS; Arm B (third top curve): Gemcitabine, 1.2mg/kg; Arm C (bottom curve): Gemcitabine 1.2 mg/kg plus anti-HDGF H3 at 12.5mg/kg; Arm D (second top curve): Gemcitabine 1.2 mg/kg plus anti-HDGF H3/VEGF trapper (H3K) at 12.5mg/kg. The drugs were given intraperitoneally every 3 days until tumor regression or sacrifice due to excess tumor burden.

[0013] FIG. 4A and FIG. 4B are two graphs illustrating bifunctional antibody-induced extended tumor (volume) remission and plots each individual tumor volume in the ten individuals treated in arm C (FIG. 4A) and arm D (FIG. 4B). Treatments were terminated after complete tumor remission in animals receiving Gemzar plus antibody; each curve represents one animal. Arm C: Gemzar plus anti-HDFG H3 treated; Arm D: Gemzar plus anti-HDGF/VEGF trapper (H3K) treated.

[0014] FIG. 5 is a graph illustrating results after treatment with bleomycin (36 ug/animal) given intratracheally to C57/B6 mice (5 – 6 weeks old, 7 per group) under anesthesia. Antibodies were given twenty-four hours later intraperitoneally in the following treatment groups: (1) control (PBS, diamonds); (2) mouse anti-HDGF H3 plus anti-HDGF C1 125 ug each (H3+C1, squares); and (3) anti-HDGF H3 250 ug plus Avastin 100 µg (H3+A, triangles). The treatments were given every 3 days thereafter for a total 6 doses, and animals were monitored daily.

[0015] FIG. 6 is a graph illustrating results from mice (5 – 6 weeks old, 7 per group) primed with antibody intraperitoneally according to the following treatment groups: (1) control (PBS, diamonds); (2) anti-HDGF H3 plus anti-HDGF C1 125 ug each (H3+C1, squares); (3) chimeric anti-HDGF H3/VEGF trapper plus anti-HDGF C1, 125 ug each (H3K + C1, triangles); and (4) chimeric anti-HDGF H3/TGFB trapper plus anti-HDGF C1, 125 ug each (h3T + C1, x). Bleomycin (36 ug/animal) was given intratracheally 24-hours later under anesthesia. Antibodies were again given on day 3 (24-hr post-bleomycin instillation), and every three days thereafter for a total of 7 doses (including priming). Animals were monitored daily.

[0016] FIG. 7A and FIG. 7B are images of high-level, sustained HDGF expression results after bleomycin treatment. FIG. 7A: Reorganization of native and post-transcriptionally modified HDGF by various anti-HDGF antibodies in A549 lung cancer cell protein extract on Western blot. FIG. 7B: Anti-HDGF antibody staining of protein extracts from Bleomycin treated lung. Bleomycin or PBS was given intratracheally to the lung of C57 mice. Lung tissue was dissected 24 or 48 hours post treatment, and homogenized in lysis buffer with

proteinase inhibitors and 1% Triton X-100. Supernatants were separated by SDS gel, blotted on nitrocellulose membrane and stained with anti-HDGF antibodies. Lanes marked 1 indicate PBS-treated lung; lanes marked 2 indicate 24 hours post bleomycin treatment; lanes marked 3 indicate 48 hours post bleomycin treatment.

[0017] FIG. 8A, FIG. 8B, FIG. 8C, and FIG. 8D are images of anti-HDGF antibody inhibiting fibrosis in bleomycin damaged lung tissue. Mice were instilled with bleomycin intratracheally; anti-HDGF H3 plus C1 were given 24 hours later. Mice then were sacrificed after one week. Lung were dissected, fixed in 4% formalin, embedded in paraffin, then 4 um sections were cut. The tissue sections were stained with hematoxylin and eosin. FIG. 8A: normal lung; FIG. 8B: Bleomycin only; FIG. 8C: Bleomycin plus PBS sham treatment; and FIG. 8D: Bleomycin plus anti-HDGF treatment.

[0018] FIG. 9 is a schematic diagram of a vector for recombinant antibody expression in mammalian cells (expression cassette of pLVBHN).

[0019] FIG. 10A and FIG. 10B are illustrations of immunoprecipitation of HDGF by humanized H3K. Humanized H3 was incubated with lung cancer cell lysate to bind HDGF. The immune complex was captured with Protein G beads and analyzed by western blotting. The lane marked HH was produced using humanized heavy chain-V trap and humanized light chains; the lane marked HM was produced using humanized heavy chain-V trap and chimeric light chain. FIG. 10A shows staining with mouse anti-HDGF H3 antibody. In FIG. 10B, shows blot re-staining with goat-anti-human IgG HRP conjugate.

[0020] FIG. 11A, FIG. 11B, and FIG. 11C are a series of three graphs showing the response of nude mice carrying human NSCLC PDX tumor MDA274 to the indicated anticancer therapies. FIG. 11A shows a summary of five treatment regimens. FIG. 11B and FIG. 11C show tumor volumes of individual mice. See Example 7.

[0021] FIG. 12A, FIG. 12B, and FIG. 12C are a series of three photographs showing MDA 2131 anti SOX2 immunohistochemical staining of treatment naïve tumor (FIG. 12A), tumor treated with Gemzar plus anti-VEGF treatment (FIG. 12B), and tumor treated with Gemzar plus H3K (FIG. 12C).

[0022] FIG. 13A, FIG. 13B, and FIG. 13C are a series of three photographs showing MDA 2131-8 anti-CD34 immunohistochemical staining of treatment naïve tumor (FIG. 13A), tumor treated with Gemzar plus anti-VEGF treatment (FIG. 13B), and tumor treated with Gemzar plus H3K (FIG. 13C).

[0023] FIG. 14A, FIG. 14B, and FIG. 14C are a series of three photographs showing MDA 2131-8 anti-CD31 immunohistochemical staining of treatment naïve tumor (FIG. 14A),

tumor treated with Gemzar plus anti-VEGF treatment (FIG. 14B), and tumor treated with Gemzar plus H3K (FIG. 14C).

[0024] FIG. 15A and FIG. 15B are diagrams showing exemplary VEGF trapper molecules.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

1. Introduction

[0025] A panel of anti-HDGF antibodies as shown in Table 1 (C1, C4, H3, cH3, hH3, H3K, hH3K, H3T, hH3T, and L5-9) has been generated in an effort to generate an antibody-based therapeutic strategy to treat hyperproliferative conditions as described herein. Treating abnormal proliferation of fibroblasts that cause keloid scarring or lung fibrosis and treating lung cancer with a novel bi-functional molecule targeting growth factors (e.g., H3K, hH3K, H3T, and hH3T) allow for a more effective simultaneous targeting of not only HDGF, but one or both of two over-produced angiogenic factors (i.e., VEGF and TGF β).

Table 1. Antibodies Targeting Growth Factors as a Strategy in Treating Hyperproliferative Conditions.

ANTIBODY	TYPE OF ANTIBODY	TARGET	PRODUCTION VEHICLE
H3	mouse monoclonal	HDGF	hybridoma cell line
cH3	chimeric	HDGF	plasmid expression clones
hH3	humanized	HDGF	plasmid expression clones
H3K	chimeric bifunctional	HDGF, VEGF-A, VEGF-C	plasmid expression clones
hH3K	humanized bifunctional	HDGF, VEGF-A, VEGF-C	plasmid expression clones
H3T	chimeric bifunctional	HDGF, TGF-beta	plasmid expression clones
hH3T	humanized bifunctional	HDGF, TGF-beta	plasmid expression clones
C1	mouse monoclonal	HDGF	hybridoma cell line
C4	mouse monoclonal	HDGF	hybridoma cell line
L5-9	mouse monoclonal	HDGF	hybridoma cell line

[0026] Therefore, embodiments are directed to a bifunctional hepatoma-derived growth factor (HDGF)-specific antibody comprising at least one complementarity determining region (CDR) specific for HDGF and at least one receptor domain that specifically binds to a growth factor selected from vascular endothelial growth factor (VEGF) or transforming growth factor beta (TGF β). In some embodiments, pharmaceutical compositions comprising the

bifunctional hepatoma-derived growth factor (HDGF)-specific antibody with a pharmaceutically acceptable carrier are provided. Further embodiments include methods reducing the growth of hyperproliferative cells in a subject by administering to the subject the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody in an amount of that down regulates HDGF and VEGF expression or HDGF and TGF β expression and is effective to reduce the abnormal growth of the hyperproliferative cells. The antibody and the bifunctional antibody did not reduce the expression of these factors in the cells that produce them, only to block the binding of these factors to cells that have receptors for these factors, e.g., fibroblasts, vascular endothelial cells, or cancer cells). Therefore, methods are provided for simultaneously reducing expression of HDGF and VEGF or HDGF and TGF β (or both) in a subject in need thereof by administering bifunctional hepatoma-derived growth factor (HDGF)-specific antibody. The subject in need suffers from a hyperproliferative condition selected from the group consisting of interstitial lung disease, keloids, lung fibrosis, idiopathic pulmonary fibrosis, lung cancer, pancreatic cancer, colon cancer, ovarian cancer, liver cancer, glioblastoma, diabetic retinopathy, age-related macular degeneration, and squamous cell carcinoma.

2. Definitions

[0027] Unless otherwise defined, all technical and scientific terms used herein are intended to have the same meaning as commonly understood in the art to which this invention pertains and at the time of its filing. Although various methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. However, the skilled should understand that the methods and materials used and described are examples and may not be the only ones suitable for use in the invention. Moreover, it should also be understood that as measurements are subject to inherent variability, any temperature, weight, volume, time interval, pH, salinity, molarity or molality, range, concentration and any other measurements, quantities or numerical expressions given herein are intended to be approximate and not exact or critical figures unless expressly stated to the contrary. Hence, where appropriate to the invention and as understood by those of skill in the art, it is proper to describe the various aspects of the invention using approximate or relative terms and terms of degree commonly employed in patent applications, such as: so dimensioned, about, approximately, substantially, essentially, consisting essentially of, comprising, and effective amount.

[0028] Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein, and nucleic acid

chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.,* Sambrook *et al.* Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lan, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Principles of Neural Science, 4th ed., Eric R. Kandel, James H. Schwartz, Thomas M. Jessell editors. McGraw-Hill/Appleton & Lange: New York, N. Y. (2000). 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.

[0029] The term "antibody" as used herein means an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative or variant of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions.

[0030] The term, "chimeric antibody (cAb)" as used herein, means an antibody wherein the variable region is derived from one species (e.g., derived from rodents) and the constant region is derived from a different species, such as human. Chimeric antibodies may be generated by antibody engineering. "Antibody engineering" is a term used generically for different kinds of modifications of antibodies, and which is a well-known process for the skilled person. In particular, a chimeric antibody may be generated by using standard DNA techniques known in the art. Thus, the chimeric may be a genetically or an enzymatically engineered recombinant antibody. It is within the knowledge of the skilled person to generate a chimeric antibody, and thus, generation of the chimeric antibody according to the present invention may be performed by other methods than described herein. Chimeric monoclonal antibodies for therapeutic applications are developed to reduce antibody immunogenicity. They may for typically contain non-human (e.g., murine) variable regions, which are specific for the antigen of interest, and human constant antibody heavy and light chain domains. The terms "variable region" or "variable domains" as used in the context of chimeric antibodies, refers to a region which comprises the CDRs and framework regions of both the heavy and light chains of the immunoglobulin. A chimeric antibody made by fusing the antigen binding region (variable domains of the heavy and light chains, VH and VL) from one species like a mouse, with the constant domain (effector region) from another

species such as a rabbit. The chimeric antibodies retain the original antibody's antigen specificity and affinity.

[0031] The term "humanized antibody" as used herein, refers to a genetically engineered non-human antibody, which contains human antibody constant domains and non-human and human variable domains modified to contain a high level of sequence homology to human variable domains. This can be achieved by grafting the antibody complementarity-determining regions (CDRs), which together form the antigen binding site, onto a homologous human acceptor framework region (FR). In order to fully reconstitute the binding affinity and specificity of the parental antibody, the substitution of framework residues from the parental antibody (i.e., the non-human antibody) into the human framework regions (back-mutations) may be required. Structural homology modeling may help to identify the amino acid residues in the framework regions that are important for the binding properties of the antibody. Thus, a humanized antibody may comprise non-human CDR sequences, primarily human framework regions optionally comprising one or more amino acid back-mutations to the non-human amino acid sequence, and fully human constant regions. Optionally, additional amino acid modifications, which are not necessarily back-mutations, may be applied to obtain a humanized antibody with preferred characteristics, such as affinity and biochemical properties. The humanized or chimeric antibody according to any aspect or embodiment of the present invention may be termed "humanized or chimeric H3 antibody" (e.g., H3K or H3T). The amino acid sequence of an antibody of non-human origin is distinct from antibodies of human origin, and therefore a non-human antibody is potentially immunogenic when administered to human patients. However, despite the non-human origin of the antibody, its CDR segments are responsible for the ability of the antibody to bind to its target antigen and humanization aims to maintain the specificity and binding affinity of the antibody. Thus, humanization of non-human therapeutic antibodies is performed to minimize its immunogenicity in man while such humanized antibodies at the same time maintain the specificity and binding affinity of the antibody of non-human origin.

[0032] The term "immunoglobulin heavy chain", "heavy chain of an immunoglobulin" or "heavy chain" as used herein is intended to refer to one of the chains of an immunoglobulin. A heavy chain is typically comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (abbreviated herein as CH) which defines the isotype of the immunoglobulin. The heavy chain constant region typically is comprised of three domains, CH1, CH2, and CH3. The heavy chain constant region may further comprise a hinge region. The term "immunoglobulin" as used herein is intended to refer to a class of

structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) low molecular weight chains and one pair of heavy (H) chains, all four potentially inter-connected by disulfide bonds. The structure of immunoglobulins has been well characterized. Within the structure of the immunoglobulin, the two heavy chains are inter-connected via disulfide bonds in the so-called "hinge region." As in the heavy chains, each light chain typically comprises several regions; a light chain variable region (abbreviated herein as VL) and a light chain constant region (abbreviated herein as CL). The light chain constant region typically comprises one domain, CL. Furthermore, the VH and VL regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL typically is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. CDR sequences may be determined by use of methods known in the art.

[0033] The term "Fab-antigen binding region" as used herein, refers to a region comprising, in the direction from the N- to C-terminal, at least a hinge region, a VL and VH region, and a CL and CH1 region. It binds to antigens, and is composed of one constant and one variable domain of each of the heavy and the light chain.

[0034] The term "Fc-effector binding region" as used herein, refers to a region comprising, in the direction from the N- to C-terminal, at least a hinge region, a CH2 region and a CH3 region. An Fc region may further comprise a CH1 region at the N-terminal end of the hinge region.

[0035] The term "hinge region" as used herein refers to the hinge region of an immunoglobulin heavy chain. Thus, for example the hinge region of a human IgG1 antibody corresponds to amino acids 216-230 according to the Eu numbering as set forth in Kabat.

[0036] The term "isotype" as used herein, refers to the immunoglobulin class (for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) or any allotype thereof, that is encoded by heavy chain constant region genes. Thus, in one embodiment, the antibody comprises a heavy chain of an immunoglobulin of the IgG1 class or any allotype thereof. Further, each heavy chain isotype can be combined with either a kappa (κ) or lambda (λ) light chain.

[0037] The term "hyperproliferative condition" as used herein refers to a condition or disorder or disease due to an abnormal proliferation of cells (e.g., endothelial cells, pericytes, smooth muscle cells). In preferred embodiments, fibroblasts, cancer cells, and vascular

endothelia cells are examples of cells.

[0038] The term, “subject” as used herein, means an organism that, including but not limited to mammals, *e.g.*, humans, dogs, cows, horses, kangaroos, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. Synonyms used herein include “patient” and “animal.”

[0039] The term, “therapeutically effective amount” as used herein means an amount of a therapeutic agent that achieves an intended therapeutic effect in a subject, *e.g.*, eliminating or reducing or mitigating the severity of a disease or condition, or a symptom of the disease or condition. The full therapeutic effect does not necessarily occur by administration of one dose and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations.

[0040] The term, “treating” as used herein means taking steps to obtain beneficial or desired results, including clinical results, such as, for example, mitigating, alleviating or ameliorating one or more symptoms of a disease; diminishing the extent of disease; delaying or slowing disease progression; ameliorating and palliating or stabilizing a metric (statistic) of disease. The effect may be prophylactic in terms of completely or partially preventing a conditions or disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a condition or disease and/or adverse effect attributable to the condition or disease.

“Treatment” refers to the steps taken. It can include any treatment of a condition or disease in a mammal, particularly in a human, and includes: (a) preventing the condition or disease or symptom thereof from occurring in a subject which may be predisposed to the condition or disease but has not yet been diagnosed as having it; (b) inhibiting the condition or disease or symptom thereof, such as, arresting its development; and (c) relieving, alleviating or ameliorating the condition or disease or symptom thereof, such as, for example causing regression of the condition or disease or symptom thereof by administering a therapeutically effective amount of the antibody.

3. Background

A. HDGF

[0041] In 1994, investigators described a novel human HDGF in Nakamura, H., et al., “Molecular cloning of complementary DNA for a novel human hepatoma-derived growth factor: its homology with high mobility group-1 protein,” J. Biol. Chem. 269: 25143-25149, 1994. HDGF was purified from conditioned medium of human hepatoma-derived cell line HuH-7. Molecular cloning of a cDNA from the cDNA library of the same cell line was done on the basis of the N-terminal amino acid sequence. The cDNA was 2.4 kb long and the

deduced amino acid sequence contained 240 amino acids without a signal peptide-like N-terminal hydrophobic sequence. By immunofluorescence study, investigators showed that HDGF is localized in the cytoplasm of hepatoma cells. Northern blots illustrated that HDGF is expressed ubiquitously in normal tissues and tumor cell lines. It was then suggested that it is a novel heparin-binding protein with mitogenic activity for fibroblasts. By PCR screening of a commercial monochromosomal hybrid panel, investigators described the gene encoding HDGF in Wanschura, S., et al., in "Mapping of the gene encoding the human hepatoma-derived growth factor (HDGF) with homology to the high-mobility group (HMG)-1 protein to Xq25," *Genomics* 32: 298-300, 1996 mapped HDGF to the X chromosome. By FISH, they refined the localization to Xq25. Subsequently, however, the International Radiation Hybrid Mapping Consortium mapped the HDGF gene to chromosome 1. Amberger, J. S. Personal Communication. Baltimore, Md. 12/11/2007 refined the localization to 1q21 based on an alignment of the HDGF sequence (GenBank D16431 with the genomic sequence (build 36.2). HDGF in *Mus musculus* bears Accession number NP_032257.

B. VEGF

[0042] Another growth factor known as VEGF was purified by Gospodarowicz *et al.* (1989) and Ferrara and Henzel (1989) from conditioned medium of bovine pituitary folliculo-stellate cells. An endothelial cell proliferation assay was used to monitor the biological activity of the protein. Vascular endothelial growth factor (VEGF), originally known as vascular permeability factor (VPF), is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. VEGF is part of a larger system that restores the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions. It has been noted that VEGF serum concentration is high in bronchial asthma and diabetes mellitus. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels.

[0043] When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply. Cancer cells that can express VEGF are able to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs such as aflibercept, bevacizumab, and ranibizumab can inhibit VEGF and control or slow those diseases.

[0044] More specifically, VEGF is a sub-family of growth factors, the platelet-derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system)

and angiogenesis (the growth of blood vessels from pre-existing vasculature). The VEGF family comprises in mammals five members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C and VEGF-D.

VEGF family	
Type	Function
VEGF-A	• Angiogenesis ○ ↑ Migration of endothelial cells ○ ↑ mitosis of endothelial cells ○ ↑ Matrix metalloproteinase activity ○ ↑ $\alpha v \beta 3$ activity ○ creation of blood vessel lumen ○ creates fenestrations • Chemotactic for macrophages and granulocytes • Vasodilation (indirectly by NO release)
VEGF-B	Embryonic angiogenesis (myocardial tissue, to be specific) ^[12]
VEGF-C	Lymphangiogenesis
VEGF-D	Needed for the development of lymphatic vasculature surrounding lung bronchioles
PIGF	Important for Vasculogenesis, Also needed for angiogenesis during ischemia, inflammation, wound healing, and cancer.

Adapted from Wikipedia

[0045] VEGF-A has been correlated with poor prognosis in breast cancer. Numerous studies show a decreased overall survival and disease-free survival in those tumors overexpressing VEGF. The overexpression of VEGF-A may be an early step in the process of metastasis, a step that is involved in the "angiogenic" switch. Although VEGF-A has been correlated with poor survival, its exact mechanism of action in the progression of tumors remains unclear. VEGF-A is also released in rheumatoid arthritis in response to $\text{TNF-}\alpha$, increasing endothelial permeability and swelling and also stimulating angiogenesis. VEGF-A is also implicated in diabetic retinopathy (DR). The microcirculatory problems in the retina of people with diabetes can cause retinal ischemia, which results in the release of VEGF-A. This can cause the creation of new blood vessels in the retina and elsewhere in the eye, heralding changes that may threaten the sight. VEGF-A plays a role in the disease pathology of the wet form age-related macular degeneration (AMD), which is the leading cause of blindness for the elderly of the industrialized world. The vascular pathology of AMD shares certain similarities with diabetic retinopathy, although the cause of disease and the typical source of

neovascularization differ between the two diseases. Once released, VEGF-A may elicit several responses. It may cause a cell to survive, move, or further differentiate. Hence, VEGF is a potential target for the treatment of cancer. The first anti-VEGF drug, a monoclonal antibody named bevacizumab, was approved in 2004. Approximately 10-15% of patients benefit from bevacizumab therapy; however, biomarkers for bevacizumab efficacy are not yet known. VEGF-D serum levels are significantly elevated in patients with angiosarcoma. Patients suffering from pulmonary emphysema have been found to have decreased levels of VEGF in the pulmonary arteries. In the kidney, increased expression of VEGF-A in glomeruli directly causes the glomerular hypertrophy that is associated with proteinuria. VEGF alterations can be predictive of early-onset pre-eclampsia.

C. TGF β

[0046] A growth factor known as transforming growth factor beta (TGF- β) is a multifunctional cytokine belonging to the transforming growth factor superfamily that includes three different isoforms (TGF- β 1 to 3, HGNC symbols TGFB1, TGFB2, TGFB3) and many other signaling proteins produced by all white blood cell lineages. Activated TGF- β complexes with other factors to form a serine/threonine kinase complex that binds to TGF- β receptors, which is composed of both type 1 and type 2 receptor subunits. After the binding of TGF- β , the type 2 receptor kinase phosphorylates and activates the type 1 receptor kinase that activates a signaling cascade. This leads to the activation of different downstream substrates and regulatory proteins, inducing transcription of different target genes that function in differentiation, chemotaxis, proliferation, and activation of many immune cells.

[0047] TGF- β is secreted by many cell types, including macrophages, in a latent form in which it is complexed with two other polypeptides, latent TGF-beta binding protein (LTBP) and latency-associated peptide (LAP). Serum proteinases such as plasmin catalyze the release of active TGF- β from the complex. This often occurs on the surface of macrophages where the latent TGF- β complex is bound to CD36 via its ligand, thrombospondin-1 (TSP-1). Inflammatory stimuli that activate macrophages enhance the release of active TGF- β by promoting the activation of plasmin. Macrophages can also endocytose IgG-bound latent TGF- β complexes that are secreted by plasma cells and then release active TGF- β into the extracellular fluid. Among its key functions is regulation of inflammatory processes, particularly in the gut.^[4] TGF- β also plays a crucial role in stem cell differentiation as well as T-cell regulation and differentiation. As such, it is a highly researched cytokine in the fields of cancer, auto-immune diseases, and infectious disease.

[0048] The TGF β superfamily includes endogenous growth inhibiting proteins; an increase in expression of TGF β often correlates with the malignancy of many cancers and a defect in the cellular growth inhibition response to TGF β . Its immunosuppressive functions then come to dominate, contributing to oncogenesis. The dysregulation of its immunosuppressive functions is also implicated in the pathogenesis of autoimmune diseases, although their effect is mediated by the environment of other cytokines present.

[0049] The primary three types are:

- TGF beta 1 — TGFB1
- TGF beta 2 — TGFB2
- TGF beta 3 — TGFB3

[0050] The peptide structures of the TGF- β isoforms are highly similar (homologies on the order of 70-80%). They are all encoded as large protein precursors; TGF- β 1 contains 390 amino acids and TGF- β 2 and TGF- β 3 each contain 412 amino acids. They each have an N-terminal signal peptide of 20-30 amino acids that they require for secretion from a cell, a pro-region called latency associated peptide (LAP), and a 112-114 amino acid C-terminal region that becomes the mature TGF- β molecule following its release from the pro-region by proteolytic cleavage. The mature TGF- β protein dimerizes to produce a 25 KDa active protein with many conserved structural motifs. TGF- β has nine cysteine residues that are conserved among its family. Eight form disulfide bonds within the protein to create a cysteine knot structure characteristic of the TGF- β superfamily. The ninth cysteine forms a disulfide bond with the ninth cysteine of another TGF- β protein to produce a dimer. Many other conserved residues in TGF- β are thought to form secondary structure through hydrophobic interactions. The region between the fifth and sixth conserved cysteines houses the most divergent area of TGF- β proteins that is exposed at the surface of the protein and is implicated in receptor binding and specificity of TGF- β .

[0051] In normal cells, TGF β , acting through its signaling pathway, stops the cell cycle at the G1 stage to stop proliferation, induce differentiation, or promote apoptosis. In many cancer cells, parts of the TGF β signaling pathway are mutated, and TGF β no longer controls the cell. These cancer cells proliferate. The surrounding stromal cells (fibroblasts) also proliferate. Both cells increase their production of TGF β . This TGF β acts on the surrounding stromal cells, immune cells, endothelial and smooth-muscle cells. It causes immunosuppression and angiogenesis, which makes the cancer more invasive. TGF β also converts effector T-cells, which normally attack cancer with an inflammatory (immune) reaction, into regulatory (suppressor) T-cells, which turn off the inflammatory reaction.

4. Overview

[0052] HDGF, VEGF, and TGF β are overexpressed in hyperproliferative conditions such as keloids, lung fibrosis and lung cancer. The overexpression correlates with aggressive biologic behaviors and poor clinical outcomes. Monoclonal antibodies specific for HDGF and chimeric and humanized versions of them (i.e., H3, C1, C4, and L5-9) have been previously developed by us and are provided in Table 1 and described in Ren, H. et al., “Antibodies targeting hepatoma-derived growth factor as a novel strategy in treating lung cancer,” *Mol Cancer Ther.* 2009 May; 8 (5): 1106-1112 and incorporated herein. Ren et al. determined that anti-HDGF was effective to inhibit tumor growth in non-small cell lung cancer xenograft models. When the monoclonal antibody H3 was combined with either bevacizumab or gemcitabine, tumor growth inhibition was enhanced. Here, chimeric and humanized bifunctional antibodies have been created (i.e., H3K, hH3K, H3T, and hH3T) to simultaneously target two additional growth factors (VEGF and TGF β) in tumor tissue. In patient-derived tumor xenograft mouse models, treatment with the bi-functional antibody H3K interfered with tumor drug resistance and demonstrated long-term remission. In mouse models of lung fibrosis, anti-HDGF antibodies in various combinations before or after bleomycin-induced damage showed therapeutic effects on survival and inhibition of fibrosis. Without being bound by theory, treatment of abnormal proliferation of fibroblasts with the bi-functional antibodies (i.e., H3K, hH3K, H3T, and hH3T) will provide an improved response in view of the simultaneous targeting of HDGF and VEGF or HDGF and TGF β . This novel molecule targeting HDGF, with a VEGF trap or TGF β trap is easier to administer as a single molecule and enhances treatment by providing equal or better effects while triggering ADCC in cancer cells. Strategies are also provided herein for reducing abnormal proliferation of fibroblasts using antibodies described in Table 1.

5. Embodiments

[0053] Therefore, embodiments of the invention include a bifunctional hepatoma-derived growth factor (HDGF)-specific antibody molecule (e.g., H3K, hH3K, H3T and hH3T) comprising at least one complementarity determining region (CDR) specific for HDGF and at least one receptor domain that specifically binds to a growth factor selected from vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF β). In other embodiments, a single chain antibody (scFv) is created and can be combined with a variety of functional moieties. In some embodiments, pharmaceutical compositions comprising the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody with a

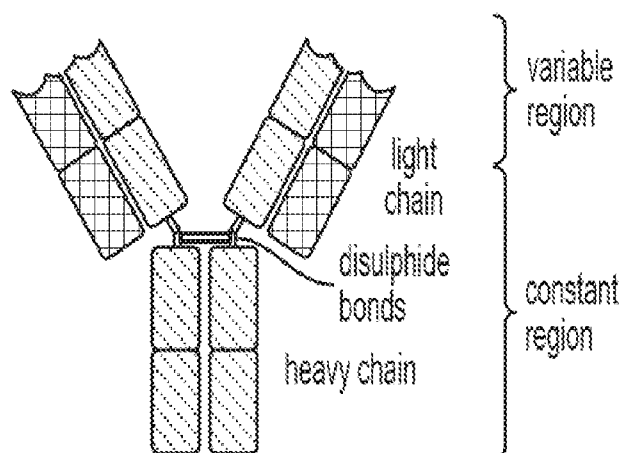
pharmaceutically acceptable carrier are provided. The pharmaceutical composition may be formulated for parenteral, intravenous or topical administration. Preferably the pharmaceutical composition is formulated for administration in an amount from 5 to 25 mg/kg every 1-3 weeks. Further embodiments include methods reducing the growth of hyperproliferative cells in a subject by administering to the subject the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody in an amount of that down regulates HDGF and VEGF expression or HDGF and TGF β expression and is effective to reduce the abnormal growth of the hyperproliferative cells. In some embodiments, the hyperproliferative cell may be a fibroblast or a cancer cell, or a vascular endothelial cell. The cancer cell may be selected from the group consisting of lung, pancreas, colon, ovarian, liver, glioblastoma, and squamous cell carcinoma. And in other embodiments, methods are provided for simultaneously reducing expression of HDGF and VEGF or HDGF and TGF β in a subject in need thereof by administering bifunctional hepatoma-derived growth factor (HDGF)-specific antibody. The subject in need suffers from a hyperproliferative condition selected from the group consisting of interstitial lung disease, keloids, lung fibrosis, idiopathic pulmonary fibrosis, lung cancer, pancreatic cancer, colon cancer, ovarian cancer, liver cancer, glioblastoma, diabetic retinopathy, age-related macular degeneration, and squamous cell carcinoma.

A. Bi-Functional Molecules and Methods of Making Them

[0054] Some embodiments of the invention include a bifunctional hepatoma-derived growth factor (HDGF)-specific chimeric or humanized antibody (e.g., H3K hH3K, H3T or hH3T) and each comprises at least one complementarity determining region (CDR) specific for HDGF and at least one receptor domain that specifically binds to a growth factor selected from vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF β). The CDR may be selected from a mouse, human, rabbit, rat, CDR, preferably a mouse CDR. The antibody may be humanized or chimeric and comprises two receptor domains (e.g., VEGFR2) each of which independently bind to a growth factor selected from vascular endothelial growth factor (VEGF-A, VEGF-C, and VEGF-E) and transforming growth factor beta (TGF β) (i.e., a TGF β selected from TGF β subtype 1, subtype 2, and subtype 3). In certain aspects the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody is linked to a reporter molecule selected from the group consisting of, an enzyme, a radiolabel, a hapten, a fluorescent label, a phosphorescent molecule, a chemiluminescent molecule, a chromophore, a luminescent molecule, a photoaffinity molecule, a ligand, a colored particle or biotin. In the alternative, the antibody is linked to an

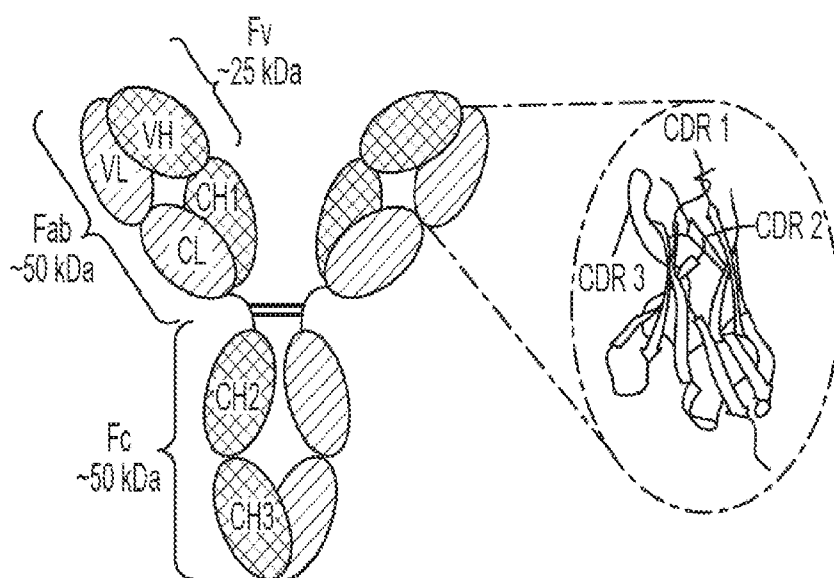
effector molecule selected from the group consisting of, a toxin, an apoptotic molecule, an antitumor agent, a therapeutic enzyme or a cytokine. In some aspects, the antitumor agent is selected from the group consisting of gemcitabine, pemetrexed, cisplatin, docetaxel, vinorelbine, doxorubicin, 6-fluorouracil, erlotinib, gefitinib, and crizotinib.

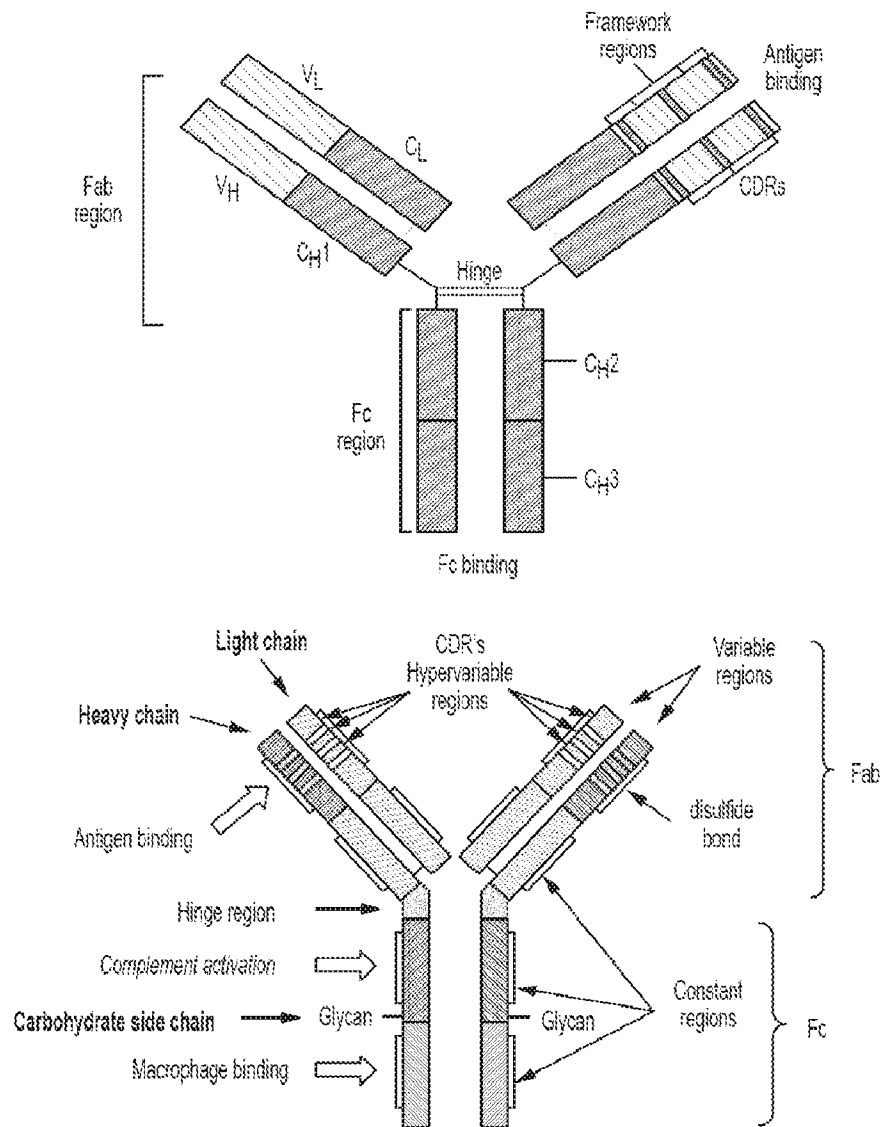
[0001] Embodiments include antibodies that are immunological proteins that bind a specific antigen. In most mammals, including humans and mice, antibodies are constructed from paired heavy and light polypeptide chains. Each chain is made up of individual immunoglobulin (Ig) domains, and thus the generic term immunoglobulin is used for such proteins. Each chain is made up of two distinct regions, referred to as the variable and constant regions. The light and heavy chain variable regions show significant sequence diversity between antibodies, and are responsible for binding the target antigen. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans there are five different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the V region. IgG antibodies are tetrameric proteins composed of two heavy chains and two light chains. The IgG heavy chain is composed of four immunoglobulin domains linked from N- to C-terminus in the order VH-CH1-CH2-CH3, referring to the heavy chain variable domain, heavy chain constant domain 1, heavy chain constant domain 2, and heavy chain constant domain 3 respectively (also referred to as VH-C γ 1-C γ 2-C γ 3, referring to the heavy chain variable domain, constant γ 1 domain, constant γ 2 domain, and constant γ 3 domain respectively). The IgG light chain is composed of two immunoglobulin domains linked from N- to C-terminus in the order VL-CL, referring to the light chain variable domain and the light chain constant domain respectively.



[0002] In some embodiments, a chimeric antibody is an antibody made by combining genetic material from a nonhuman source, like a mouse, with genetic material from a human being. Chimeric antibodies are generally around two thirds human, reducing the risk of a reaction to foreign antibodies from a non-human animal when they are used in therapeutic treatments. A closely related concept is a humanized antibody, made in a similar way but containing closer to 90% human genetic material. Using recombinant technology, people can cut and splice genetic material from multiple sources and fuse it together. A chimeric antibody contains antibodies developed with animal cells in culture, with sections of the genetic code replaced with human genes in order to address concerns about a potential reaction with the animal's genetic material.

[0003] The variable region of the Fab-antigen binding region of antibodies in some embodiments contains the antigen binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The variable region is so named because it is the most distinct in sequence from other antibodies within the same class. The majority of sequence variability occurs in the complementarity determining regions (CDRs). There are 6 CDRs total, three each per heavy and light chain, designated VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3. In preferred embodiments, the CDR is mouse CDR for HDGF.





[0004] In preferred embodiments, the CDR is a mouse CDR for HDGF. The variable region outside of the CDRs is referred to as the framework (FR) region. Although not as diverse as the CDRs, sequence variability does occur in the FR region between different antibodies. Overall, this characteristic architecture of antibodies provides a stable scaffold (the FR region) upon which substantial antigen binding diversity (the CDRs) can be explored by the immune system to obtain specificity for a broad array of antigens. A number of high-resolution structures are available for a variety of variable region fragments from different organisms, some unbound and some in complex with antigen. The sequence and structural features of antibody variable regions are well characterized (Morea et al., 1997, *Biophys Chem* 68:9-16; Morea et al., 2000, *Methods* 20:267-279, entirely incorporated by reference), and the conserved features of antibodies have enabled the development of a wealth of antibody engineering techniques (Maynard et al., 2000, *Annu Rev Biomed Eng* 2:339-376, entirely incorporated by reference). For example, it is possible to graft the CDRs from one

antibody, for example a murine antibody, onto the framework region of another antibody, for example a human antibody. This process, referred to in the art as "humanization," reduces the immunogenicity of antibody therapeutics compared to nonhuman antibodies. Fragments including the variable region can exist in the absence of other regions of the antibody, including for example the antigen binding fragment (Fab) including VH-C.gamma.1 and VH-CL, the variable fragment (Fv) including VH and VL, the single chain variable fragment (scFv) including VH and VL linked together in the same chain, as well as a variety of other variable region fragments (Little et al., 2000, *Immunol Today* 21:364-370, entirely incorporated by reference).

[0059] A Fab fragment is a monovalent fragment consisting of the VL, VH, CL and CH I domains; a F(ab')₂ fragment is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consists of the VH and CH1 domains; an Fv fragment consists of the VL and VH domains of a single arm of an antibody; and a dAb fragment (Ward et al., *Nature* 341:544 546, 1989) consists of a VH domain. A single-chain antibody (scFv) is an antibody in which a VL and VH regions are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain (Bird *et al.*, *Science* 242:423 426, 1988 and Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879 5883, 1988). Such linker molecules are commonly known in the art and described in U.S. Patent Application No. 20130245233 A1, Denardo et al., (1998) *Clin Cancer Res.* 4(10):2483-90; Peterson et al., (1999) *Bioconjug. Chem.* 10(4):553-7; and Zimmerman et al., (1999) *Nucl. Med. Biol.* 26(8):943-50, each incorporated by reference in their entireties. Diabodies are bivalent, bispecific, or bi-functional antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (*see e.g.*, Holliger, P., *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444 6448, 1993, and Poljak, R. J., *et al.*, *Structure* 2:1121 1123, 1994). One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it bispecific.

[0060] An Fc-effector binding region is a fragment containing a constant region, termed the Fc domain, which engages a diversity of cellular receptors, thereby triggering antibody-mediated effector functions. The Fc domain acts as a bridge between the specificity dictated by the Fab region and cells of the innate and adaptive immune system. A notable common characteristic of both classes of therapeutic antibodies is the importance of the IgG Fc domain, which connects the fine specificity of an antibody with immune cells that mediate

antibody-triggered effector functions through their engagement of Fc receptor (FcR) family members. In antibody dependent cellular cytotoxicity (ADCC), FcγRs on the surface of effector cells (natural killer cells, macrophages, monocytes and eosinophils) bind to the Fc region of an IgG which itself is bound to a target cell. Engineering the antibody Fc region to enhance the cytotoxic activity of therapeutic antibodies is preferred in some embodiments. In preferred embodiments, the Fc-effort region comprises a human VEGF/TGFβ trap. The growth factor receptor dimers bind GF dimer. Fusion with the heavy chain creates a receptor dimer which may be required or enhance the binding of GF.

[0061] In preferred embodiments, anti-HDGF hybridoma Balb/c mice were immunized with recombinant HDGF in Freund's adjuvant. Splenocytes from immunized animals were fused with P3x63Ag8.653 cells and screened for HDGF reactivity in the culture supernatant. Positive clones were identified and anti-HDGF antibody secretion was verified by immunoblot analysis of cancer cell lysate and purified HDGF. RNA from anti-HDGF antibody producing hybridoma was extracted, reverse transcribed into cDNA. Primers designed to amplify the Ig variable region (Larrick, J.W., et al. 1989. Biochem. Biophys. Res. Comm. 160, 1250. Jones, S.T. and Bendig, M.M. 1991. Biotechnology 9, 88) were used to amplify the cDNA encoding the mouse Ig heavy and light chain. The amplified product were cloned and sequenced. The cDNA sequence that encoded the murine Ig heavy chain and light chain, and the CDRs were identified as described in <http://www.bioinf.org.uk/abs/>. The DNA fragments that encoded the murine antibody variable domain (VH and VL) were grafted to the N-terminal of the constant region of their human IgG1 constant part by standard molecular biology techniques, and cloned into a mammalian expression vector respectively. To produce the chimeric antibody, plasmids encoding for the heavy and light chain were co-transfected into Expi293 cells (Invitrogen®) at a ratio of 1:2. The antibody was purified from the conditioned media by protein G affinity chromatograph. The cDNA sequence that encoded the murine Ig heavy chain and light chain, and the CDRs were identified as described by Martin A.C.R. (<http://www.bioinf.org.uk/abs/>). The humanized anti-HDGF Vh sequences were then created by replacing the CDRs in human Ig G1 Vh with the corresponding murine CDRs. The DNA sequence that encode the humanized sequence is then synthesized and grafted on the N-termini of the human IgG1 heavy chain constant region, cloned into a mammalian expression vector to generate a humanized Ig heavy chain expression construct. The humanized VL expression construct was created similarly. To produce the humanized anti-HDGF antibody, the plasmids that encode for the heavy and light

chain was used to transfect Expi293 cell at a ratio of 1:2. The antibody was purified from the conditioned media by protein G affinity chromatograph.

[0062] Construction of Fc-fused growth factor binding domain (GF trapper) included a DNA fragment corresponding to domain 2 and 3 (D2 and D3) of human VEGF receptor 2 (kinase insert domain receptor) amino acid residue 122 to residue 327 (based on NP_002253) and was amplified from HEK 293 cDNA. The amplified sequence was fused to the C-terminus of the human IgG1 heavy chain via a poly-GlySer linker, (GlyGlyGlyGlySer)₂, or (G4S)₂. The full-length anti-HDGF antibody heavy chain-VEGF trapper sequence was cloned into a mammalian expression plasmid.

[0063] Construction of Fc-TGFB trapper included a DNA fragment corresponding to human TGFBR2 extracellular domain amino acid residue 27 to residue 184 (based on NP_001020018) was amplified from human lung cDNA. The amplified sequence was fused to the C-terminus of the human IgG1 heavy chain via a poly-GlySer linker, (GlyGlyGlyGlySer)₂, or (G4S)₂. The full-length anti-HDGF antibody heavy chain-TGFB trapper sequence was cloned into a mammalian expression plasmid. To produce the recombinant anti-HDGF antibody, plasmids encoding for the heavy and light chain were co-transfected into Expi293 cells (Invitrogen®) at a ratio of 1:2. The antibody was purified from the conditioned media by protein G affinity chromatograph.

[0064] Chimeric antibodies may be generated by substituting all constant region sequences of a non-human (such as murine) antibody with constant region sequences of human origin or vice versa. Thus, fully non-human variable region sequences are maintained in the chimeric antibody. Thus, a chimeric antibody according to the present invention may be produced by a method comprising the step of expressing the non-human variable heavy chain, non-human variable light chain sequences, human constant heavy chain and human constant light chain sequences in suitable expression systems, and thereby generating a full-length chimeric antibody. Alternative methods may be used. Such methods of producing a chimeric antibody is within the knowledge of the skilled person, and thus, the skilled person would know how to produce a chimeric antibody according to the present invention.

[0065] The term "recombinant human antibody," as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes, (b) antibodies expressed using a recombinant expression vector transfected into a host cell, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (c) antibodies prepared, expressed, created or isolated by any other

means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies preferably have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. Antigen-binding portions include, *inter alia*, Fab, Fab', F(ab')₂, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the target ceramide. Also included in the definition of antibodies is the SuperAntibody including those chemically conjugated to T15 peptide or genetically-engineered into a human IgG1 backbone (see Y. Zhao, D. Lou, J. Burkett and H. Kohler. Enhanced Anti-B-cell Tumor Effects with Anti-CD20 SuperAntibody. *J. Immunotherapy*, 25: 57-62, 2002). The immunoglobulin subtype can be any subtype; typically IgG and IgM are used, but IgA, IgE etc. also can be effective.

[0066] In preferred embodiments, the chimeric antibody may have one or more binding sites and is a bifunctional hepatoma-derived growth factor (HDGF)-specific chimeric or humanized antibody (e.g., H3K hH3K, H3T or hH3T). If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a "bispecific" or "bifunctional" antibody has two different binding sites.

[0067] In some embodiments, the carboxy termini of the chimeric H3 heavy chain is fused to a soluble VEGF receptor (VEGF trapper). The VEGF trapper preferably is made by fusing the VEGF receptor 2 (VEGFR2) ligand binding domain with another protein at the carboxyl terminal via a linker. In this instance, the ligand binding domain consists of sequences from D2 and D3 of VEGFR2; the linker sequence here is a tandem repeat of G4S, but other types of linker, such as a polypeptide or synthetic linker, are possible. In some embodiments, the VEGF trapper is constructed using the ligand binding domain of VEGFR1, VEGFR2 or VEGFR3; or a hybrid ligand binding domain consisting of a D2 domain selected from VEGFR1, VEGFR2 or VEGFR3, and a D3 domain selected from VEGFR1, VEGFR2 or VEGFR3; or a hybrid ECD consisting of the fusion of a D2 domain containing ECD and a

D3 domain containing ECD. See FIG. 15A and 15B for examples of VEGF trapper constructions.

[0068] Without being bound by theory, the majority of the methods of making a soluble receptor derived from type I trans membrane protein/ type I receptor is to fuse the N-terminal extracellular domain to the N-termini of Fc fragment due to: 1) the natural topology of the receptor protein, 2) the requirement of many of these receptors to dimerize upon binding the ligand which is often dimerized, 3) favorable PK due to similarity to antibody. Although expression as Fc fusion protein is the easiest way, it is possible to make multi-functional molecule or nanoparticle by covalent or non-covalent conjugation of VEGFR monomer or dimer, as long as it can form a dimer before or upon GF dimer binding.

[0069] The VEGF trapper has an amino acid sequence set forth in SEQ ID NO:1.

Embodiments including a VEGF trapper preferably have the VEGF trapper fused at the carboxyl termini of the human IgG heavy chain. In the following example, a human IgG1 heavy chain constant region and the VEGF binding domain are shown in SEQ ID NO:2. In other embodiments, the VEGF trapper was fused at the carboxyl termini of a chimeric H3 heavy chain. In the following example, a human IgG1 heavy chain constant region and VEGF binding domain is shown in SEQ ID NO:3. The VEGF trapper alternatively was fused at the carboxyl termini of a humanized anti-HDGF H3 heavy chain (matured peptide) in other embodiments as shown in SEQ ID NO: 4. In this instance, the TGFB trapper is shown as a carboxyl-terminal fusion via a linker in SEQ ID NO:5. The linker is (G4S)₂. Other alternative embodiments include a TGFB trapper fused at the carboxyl termini of human IgG heavy chain (SEQ ID NO:6). In a further alternative embodiment, the TGFB trapper was fused at the carboxyl termini of chimeric anti-HDGF H3 heavy chain (SEQ ID NO:7). In other embodiments, the TGFB trapper was fused at the carboxyl termini of humanized anti-HDGF H3 heavy chain (matured peptide) (SEQ ID NO:8).

B. Biologically Active Fragments and Variants

[0070] As indicated above, the term “antibody” as used herein, unless otherwise stated or clearly contradicted by context, includes any fragment of an antibody that retains the ability to specifically interact, such as bind, to the antigen. It has been shown that the antigen-binding function of an antibody may be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antibody” include (i) a Fab' or Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains, or a monovalent antibody as described in WO2007059782 (Genmab A/S); (ii) F(ab')₂ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge

region; (iii) a Fd fragment consisting essentially of the V_H and C_{H1} domains; and (iv) a Fv fragment consisting essentially of the V_L and V_H domains of a single arm of an antibody. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988)). Such single chain antibodies are encompassed within the term antibody unless otherwise noted or clearly indicated by context. Although such fragments are generally included within the meaning of antibody, they collectively and each independently are unique features of the present invention, exhibiting different biological properties and utility. These and other useful antibody fragments in the context of the present invention are discussed further herein. It also should be understood that the term antibody, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies and humanized antibodies, and antibody fragments retaining the ability to specifically bind to the antigen (antigen-binding fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. An antibody as generated can possess any isotype.

[0071] “Biologically active fragments” of an antibody as used herein, mean any fragment that retains binding affinity for HDGF. The fragments retain one or more CDR regions from the original antibody. CDRs are the sites of the antibody that bind to the antigen and in most cases are unique to that antibody. In order for a fragment to retain binding to the antigen, it would need to have at a set of CDRs with 3D structure such that they are combined to form a binding pocket or the like. Biologically active fragments may also contain minor variations provided that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99% sequence identity and the molecule retains its affinity for binding.

[0072] “Variants” of a bi-functional chimeric antibody or fragment thereof include amino acid sequence modification(s) of the antibodies described herein that may, for example, improve the binding affinity and/or other biological properties of the antibody for the intended purpose of treating or mitigating an enumerated disease. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues

within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired affinity for HDGF. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

[0073] Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al.* Science 253:164 (1991).

C. Pharmaceutical Compositions

[0074] In some embodiments, pharmaceutical compositions comprising the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody with a pharmaceutically acceptable carrier are provided. The pharmaceutical composition may be formulated for parenteral, intravenous or topical administration. Preferably the pharmaceutical composition is formulated for administration in an amount from 5 to 25 mg/kg every 1-3 weeks. The pharmaceutical compositions may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques known in the art.

[0075] The pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients should be suitable for the humanized or chimeric antibodies of the present invention and the chosen mode of administration. Suitability for carriers and other components of pharmaceutical compositions is determined based on the lack of significant negative impact on the desired biological properties of the chosen compound or pharmaceutical composition of the present invention (e.g., less than a substantial impact (10% or less relative inhibition, 5% or less relative inhibition, etc.)) on antigen binding.

[0076] Pharmaceutically acceptable carriers include any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonicity agents, antioxidants and absorption-delaying agents, and the like that are physiologically compatible with a humanized or chimeric antibody of the present invention. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the

present invention include water, saline, phosphate buffered saline, ethanol, dextrose, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, corn oil, peanut oil, cottonseed oil, and sesame oil, carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate, and/or various buffers. Other carriers are well known in the pharmaceutical arts.

[0077] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present invention is contemplated. When referring to the "active compound" it is contemplated to also refer to the humanized or chimeric antibody according to the present invention.

[0078] Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0079] A pharmaceutical composition of the present invention may also include diluents, fillers, salts, buffers, detergents (e.g., a nonionic detergent, such as Tween-20 or Tween-80), stabilizers (e.g., sugars or protein-free amino acids), preservatives, tissue fixatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition.

[0080] The actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0081] The pharmaceutical composition may be administered by any suitable route and mode. Suitable routes of administering a humanized or chimeric antibody of the present

invention in vivo and in vitro are well known in the art and may be selected by those of ordinary skill in the art.

[0082] In one embodiment, a pharmaceutical composition of the present invention is administered parenterally. The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intra-orbital, intracardiac, intradermal, intraperitoneal, intratendinous, local injection, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion.

[0083] In one embodiment that pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion. The pharmaceutical compositions of the present invention may also contain one or more adjuvants appropriate for the chosen route of administration such as preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the pharmaceutical composition. The humanized or chimeric antibody of the present invention may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and micro-encapsulated delivery systems. Such carriers may include gelatin, glyceryl monostearate, glyceryl distearate, biodegradable, biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, poly-orthoesters, and polylactic acid alone or with a wax, or other materials well known in the art. Methods for the preparation of such formulations are generally known to those skilled in the art.

[0084] In one embodiment, the humanized or chimeric antibody of the present invention may be formulated to ensure proper distribution in vivo. Pharmaceutically acceptable carriers for parenteral administration include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present invention is contemplated. Other active or therapeutic compounds may also be incorporated into the compositions.

[0085] Pharmaceutical compositions for injection must typically be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, micro-emulsion, liposome, or other ordered structure suitable to high drug concentration.

The carrier may be an aqueous or a non-aqueous solvent or dispersion medium containing for instance water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. The proper fluidity may be maintained, for example, by the use of an emulsifier such as lecithin that can be applied as a coating, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as glycerol, mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients e.g., as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g., from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0086] Sterile injectable solutions may 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 sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that 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, examples of methods of preparation are vacuum-drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

D. Therapeutic Applications

[0087] Chimeric antibodies have been developed for therapeutic use. Representative publications related to such therapies include Chamow et al., 1996, Trends Biotechnol. 14:52-60; Ashkenazi et al., 1997, Curr. Opin. Immunol. 9:195-200, Cragg et al., 1999, Curr. Opin. Immunol. 11:541-547; Glennie et al., 2000, Immunol. Today 21:403-410, McLaughlin et al., 1998, J. Clin. Oncol. 16:2825-2833, and Cobleigh et al., 1999, J. Clin. Oncol. 17:2639-2648,

all entirely incorporated by reference. Currently for anticancer therapy, any small improvement in mortality rate defines success. Certain IgG variants disclosed herein enhance the capacity of antibodies to limit further growth or destroy at least partially, targeted cancer cells.

[0088] Anti-tumor potency of antibodies is via enhancement of their ability to mediate cytotoxic effector functions such as ADCC, ADCP, and CDC. Examples include Clynes et al., 1998, Proc. Natl. Acad. Sci. USA 95:652-656; Clynes et al., 2000, Nat. Med. 6:443-446 and Cartron et al., 2002, Blood 99:754-758, both entirely incorporated by reference.

[0089] Human IgG1 is the most commonly used antibody for therapeutic purposes, and the majority of engineering studies have been constructed in this context. The different isotypes of the IgG class however, including IgG1, IgG2, IgG3, and IgG4, have unique physical, biological, and clinical properties. There is a need in the art to design improved IgG1, IgG2, IgG3, and IgG4 variants. There is a further need to design such variants to improve binding to FcRn and/or increase in vivo half-life as compared to native IgG polypeptides. The present application meets these and other needs.

[0090] Further embodiments include methods reducing the growth of hyperproliferative cells in a subject by administering to the subject the bifunctional hepatoma-derived growth factor (HDGF)-specific antibody in an amount of that down regulates HDGF and VEGF expression or HDGF and TGF β expression or both VEGF and TGF β expression and is effective to reduce the abnormal growth of the hyperproliferative cells. It is possible to co-administer a mixture of these antibodies (i.e., H3K + H3T) or with other antibody-based drugs such as Cetuximab or Herceptin. In some embodiments, the hyperproliferative cell may be a fibroblast, or a cancer cell, or a vascular endothelia cell. The cancer cell may be selected from the group consisting of lung, pancreas, colon, ovarian, liver, glioblastoma, and squamous cell carcinoma.

[0091] In some embodiments, methods are provided for simultaneously reducing expression of HDGF and VEGF or HDGF and TGF β in a subject in need thereof by administering bifunctional hepatoma-derived growth factor (HDGF)-specific antibody. The subject in need suffers from a hyperproliferative condition selected from the group consisting of interstitial lung disease, keloids, lung fibrosis, idiopathic pulmonary fibrosis, lung cancer, pancreatic cancer, colon cancer, ovarian cancer, liver cancer, glioblastoma, diabetic retinopathy, age-related macular degeneration, and squamous cell carcinoma.

[0092] In another aspect, the present invention relates to a humanized or chimeric antibody, or pharmaceutical composition of the invention as defined in any aspect or embodiment herein described, for use in the treatment of a disease.

[0093] The humanized or chimeric antibody or pharmaceutical composition of the invention can be used as in the treatment of any cancer. For example, the humanized or chimeric antibody may be administered to cells in culture, e.g., in vitro or ex vivo, or to human subjects, e.g., in vivo, to treat or prevent disorders such as cancer, inflammatory or autoimmune disorders. As used herein, the term "subject" is typically a human which respond to the humanized or chimeric antibody, or pharmaceutical composition. Subjects may for instance include human patients having disorders that may be corrected or ameliorated by modulating a target function or by leading to killing of the cell, directly or indirectly.

[0094] In another aspect, the present invention provides methods for treating or preventing a hyperproliferative condition, such as keloids or lung fibrosis or diabetic retinopathy, or age-related macular degeneration, which method comprises administration of a therapeutically effective amount of a humanized or chimeric antibody, or pharmaceutical composition of the present invention to a subject in need thereof. The method typically involves administering to a subject a humanized or chimeric antibody in an amount effective to treat or prevent the disorder.

[0095] In one particular aspect, the present invention relates to a method of treatment of lung cancer comprising administering the humanized or chimeric antibody or pharmaceutical composition of the invention as defined in any aspect and embodiments herein described, to a subject in need thereof.

[0096] In another aspect, the present invention relates to the use or the method as defined in any aspect or embodiments herein described wherein the humanized or chimeric antibody is a bi-functional antibody specifically binding to both VEGF or TGF β or both and a cancer-specific target, or a target that is overexpressed in cancer or associated with cancer, such as HDGF and wherein the disease is cancer, such as breast cancer, prostate cancer, non-small cell lung cancer, bladder cancer, ovarian cancer, gastric cancer, colorectal cancer, esophageal cancer and squamous cell carcinoma of the head & neck, cervical cancer, pancreatic cancer, testis cancer, malignant melanoma, a soft-tissue cancer (e.g., synovial sarcoma), an indolent or aggressive form of B-cell lymphoma, chronic lymphatic leukemia or acute lymphatic leukemia.

E. Dosage and Administration

[0097] The efficient dosages and dosage regimens for the humanized or chimeric antibody depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[0098] A physician having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician could start doses of the humanized or chimeric antibody employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable dose of a composition of the present invention will be that amount of the humanized or chimeric antibody which is the lowest dose effective to produce a therapeutic effect according to a particular dosage regimen. Such an effective dose will generally depend upon the factors described above.

[0099] For example, an "effective amount" for therapeutic use may be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may, for example, be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition may be evaluated by examining the ability of the humanized or chimeric antibody to inhibit cell growth or to induce cytotoxicity by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound, i.e., a therapeutic humanized or chimeric antibody, or pharmaceutical composition according to the invention, may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[0100] An exemplary, non-limiting range for a therapeutically effective amount of a humanized or chimeric antibody of the invention is about 0.001-30 mg/kg, such as about 0.001-20 mg/kg, such as about 0.001-10 mg/kg, such as about 0.001-5 mg/kg, for example about 0.001-2 mg/kg, such as about 0.001-1 mg/kg, for instance about 0.001, about 0.01, about 0.1, about 1, about 5, about 8, about 10, about 12, about 15, or about 18 mg/kg.

[0101] Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

[0102] In one embodiment, the efficacy of the treatment is monitored during the therapy, e.g., at predefined points in time.

[0103] If desired, an effective daily dose of a pharmaceutical composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In another embodiment, the humanized or chimeric antibody, or pharmaceutical composition is administered by slow continuous infusion over a long period, such as more than 24 hours, in order to minimize any unwanted side effects.

[0104] While it is possible for a humanized or chimeric antibody of the present invention to be administered alone, it is preferable to administer the humanized or chimeric antibody with a pharmaceutically acceptable carrier as a pharmaceutical composition as described above.

[0105] An effective dose of a humanized or chimeric antibody of the invention may also be administered using a weekly, biweekly or triweekly dosing period. The dosing period may be restricted to, e.g., 8 weeks, 12 weeks or until clinical progression has been established.

Alternatively, an effective dose of a humanized or chimeric antibody of the invention may be administered every second, third or fourth week. Preferred administration is 5-25 mg/kg every 1-3 weeks.

[0106] In one embodiment, the humanized or chimeric antibody may be administered by infusion in a weekly dosage of calculated by mg/m^2 . Such dosages can, for example, be based on the mg/kg dosage. Such administration may be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours. In one embodiment, the humanized or chimeric antibody may be administered by slow continuous infusion over a long period, such as more than 24 hours, in order to reduce toxic side effects.

[0107] In one embodiment, the humanized or chimeric antibody may be administered in a weekly dosage of calculated as a fixed dose for up to 8 times, such as from 4 to 6 times when given once a week. Such regimen may be repeated one or more times as necessary, for example, after 6 months or 12 months. Such fixed dosages can, for example, be based on the mg/kg dosages provided above, with a body weight estimate of 70 kg. The dosage may be determined or adjusted by measuring the amount of humanized or chimeric antibody of the present invention in the blood upon administration by for instance taking out a biological sample and using anti-idiotypic antibodies which target the binding region of the humanized or chimeric antibodies of the present invention.

[0108] In one embodiment, the humanized or chimeric antibody may be administered by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

[0109] A humanized or chimeric antibody may also be administered prophylactically in order to reduce the risk of developing cancer, delay the onset of the occurrence of an event in cancer progression, and/or reduce the risk of recurrence when a cancer is in remission.

[0110] Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0111] Pharmaceutical compositions comprising a humanized or chimeric antibody according to the present invention will be formulated as solutions, suspensions, or other dosage forms for topical ophthalmic administration in a pharmaceutically acceptable carrier, adjuvant, or vehicle for treatment of ocular condition such as diabetic retinopathy and age-related macular degeneration. Preferred embodiments include aqueous solutions due to their ease of formulation, as well as a subject's ability to easily administer these compositions to the eye via one to two drops of the solution in the affected eyes. The compositions, however, may also be suspensions, viscous or semi-viscous gels, or other types of solid or semi-solid compositions. Additional ingredients that may be included in the formulation include carriers, tonicity enhancers, preservatives, solubilizers, non-toxic excipients, demulcents, sequestering agents, pH adjusting agents, co-solvents, and viscosity binding agents.

[0112] A humanized or chimeric antibody may also be administered prophylactically in order to reduce the risk of developing a hyperproliferative condition (e.g., lung fibrosis, keloids, interstitial lung disease, and lung cancer), delay the onset of the occurrence of an event in cancer progression, and/or reduce the risk of recurrence when a cancer is in remission. This may be especially useful in patients wherein it is difficult to locate a tumor that is known to be present due to other biological factors.

F. Diagnostic Applications

[0113] The humanized or chimeric antibody of the invention may also be used for diagnostic purposes, using a composition comprising a humanized or chimeric antibody as

described herein. Accordingly, the invention provides diagnostic methods and compositions using the humanized or chimeric antibodies described herein. Such methods and compositions can be used for purely diagnostic purposes, such as detecting or identifying a disease, as well as for monitoring of the progress of therapeutic treatments, monitoring disease progression, assessing status after treatment, monitoring for recurrence of disease, evaluating risk of developing a disease, and the like.

[0114] In one aspect, the humanized or chimeric antibody of the present invention is used *ex vivo*, such as in diagnosing a disease in which cells expressing a specific target of interest and to which the humanized or chimeric antibody binds, are indicative of disease or involved in the pathogenesis, by detecting levels of the target or levels of cells which express the target of interest on their cell surface in a sample taken from a patient. This may be achieved, for example, by contacting the sample to be tested, optionally along with a control sample, with the humanized or chimeric antibody according to the invention under conditions that allow for binding of the antibody to the target. Complex formation can then be detected (e.g., using an ELISA). When using a control sample along with the test sample, the level of humanized or chimeric antibody or antibody-target complex is analyzed in both samples and a statistically significant higher level of humanized or chimeric antibody or antibody-target complex in the test sample indicates a higher level of the target in the test sample compared with the control sample.

[0115] Examples of conventional immunoassays in which humanized or chimeric antibodies of the present invention can be used include, without limitation, ELISA, RIA, FACS assays, plasmon resonance assays, chromatographic assays, tissue immunohistochemistry, western blot, and/or immunoprecipitation, immunofluorescent assay methods including microarrays.

[0116] Accordingly, in one embodiment, the present invention relates to a method of diagnosing a disease characterized by involvement or accumulation of HDGF-expressing cells, comprising administering an antibody, bispecific antibody, composition or pharmaceutical composition according to any aspect or embodiment herein described, to a subject, optionally wherein the antibody is labeled with a detectable label.

[0117] In one embodiment, the invention relates to a method for detecting the presence of a target, or a cell expressing the target, in a sample comprising: contacting the sample with a humanized or chimeric antibody of the invention under conditions that allow for binding of the humanized or chimeric antibody to the target in the sample; and analyzing whether a complex has been formed. Typically, the sample is a biological sample.

[0118] In one embodiment, the sample is a tissue sample known or suspected of containing a specific target and/or cells expressing the target, a non-tissue sample or fluid with or without cells. For example, in situ detection of the target expression may be accomplished by removing a histological specimen from a patient, and providing the humanized or chimeric antibody of the present invention to such a specimen. The humanized or chimeric antibody may be provided by applying or by overlaying the humanized or chimeric antibody to the specimen, which is then detected using suitable means. It is then possible to determine not only the presence of the target or target-expressing cells, but also the distribution of the target or target-expressing cells in the examined tissue (e.g., in the context of assessing the spread of cancer cells). Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) may be modified in order to achieve such in situ detection.

[0119] In the above assays, the humanized or chimeric antibody can be labeled with a detectable substance to allow bound antibody to be detected. Alternatively, bound (primary) specific humanized or chimeric antibody may be detected by an antibody which is labeled with a detectable substance and which binds to the primary specific humanized or chimeric antibody. Furthermore, in the above assays, a diagnostic composition comprising an antibody or bispecific antibody according to any aspect or embodiments herein described may be used. Thus, in one aspect, the present invention relates to a diagnostic composition comprising an antibody or bispecific antibody according to any aspect or embodiment herein described.

[0120] Suitable labels for the target-specific humanized or chimeric antibody, secondary antibody and/or target standard used in in vitro diagnostic techniques include, without limitation, various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, .beta.-galactosidase, and acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dan syl chloride and phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S , and ^3H .

G. Kits

[0121] The invention also provides kits (or articles of manufacture) for use in the treatment of the disorders described above. Kits of the invention include one or more containers

comprising a purified engineered polypeptide conjugate and instructions for using the conjugate for treating a disease. For example, the instructions comprise a description of administration of the engineered polypeptide conjugate to treat a disease, such as cancer (e.g., colon, esophageal, gastric, head and neck, lung, ovarian, or pancreatic cancer). The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the disease and the stage of the disease.

[0122] The instructions relating to the use of the engineered polypeptide conjugate generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or subunit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

[0123] The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an engineered polypeptide as described herein. The container may further comprise a second pharmaceutically active agent.

[0124] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

[0125] Thus, in one aspect, the present invention provides a kit for detecting the presence of a growth factor (HDGF, VEGF, and TGF β) in a sample comprising the steps of;

a) contacting the sample with an antibody or bispecific antibody according to the invention, under conditions that allow for formation of a complex between the antibody or bifunctional antibody and the target of interest; and b) analyzing whether a complex has been formed. In some embodiments, anti-human or anti mouse IgG antibodies may be used to detect binding. It is possible to also use the antibody in certain types of array or micro-fluidic device which

can use electron-optical method to detect the binding. An example of this is surface plasmon resonance or other methods based on surface interaction and optical or surface atomic force scanning.

[0126] In one embodiment, the present invention provides a kit for diagnosis of cancer comprising a container comprising a target-specific humanized or chimeric antibody, and one or more reagents for detecting binding of the target-specific humanized or chimeric antibody to the target. Reagents may include, for example, fluorescent tags, enzymatic tags, or other detectable tags. The reagents may also include secondary or tertiary antibodies or reagents for enzymatic reactions, wherein the enzymatic reactions produce a product that may be visualized. In one embodiment, the present invention provides a diagnostic kit comprising one or more target-specific humanized or chimeric antibodies of the present invention in labeled or unlabeled form in suitable container(s), reagents for the incubations for an indirect assay, and substrates or derivatizing agents for detection in such an assay, depending on the nature of the label. Control reagent(s) and instructions for use also may be included.

[0127] Diagnostic kits may also be supplied for use with a target-specific humanized or chimeric antibody, such as a labeled target-specific antibody, for the detection of the presence of the target in a tissue sample or host. In such diagnostic kits, as well as in kits for therapeutic uses described elsewhere herein, a target-specific humanized or chimeric antibody typically may be provided in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for a target cell or peptide. Typically, a pharmaceutically acceptable carrier (e.g., an inert diluent) and/or components thereof, such as a Tris, phosphate, or carbonate buffer, stabilizers, preservatives, biocides, inert proteins, e.g., serum albumin, or the like, also are included (typically in a separate container for mixing) and additional reagents (also typically in separate container(s)). In certain kits, a secondary antibody capable of binding to the target-specific humanized or chimeric antibody, which typically is present in a separate container, is also included. The second antibody is typically conjugated to a label and formulated in a manner similar to the target-specific humanized or chimeric antibody of the present invention. Using the methods described above and elsewhere herein, target-specific humanized or chimeric antibodies may be used to define subsets of cancer/tumor cells and characterize such cells and related tumor tissues.

H. Therapeutic Agents

[0128] Many therapeutic agents are available that can be conjugated to an antibody or used in conjunction with antibody therapies described herein. In embodiments, therapeutic agents, including, but not limited to, cytotoxic agents, anti-angiogenic agents, pro-apoptotic agents,

antibiotics, hormones, hormone antagonists, chemokines, drugs, prodrugs, toxins, enzymes or other agents may be used as adjunct therapies when using the antibody/peptide ligand complexes described herein. Drugs useful in the invention may, for example, possess a pharmaceutical property selected from the group consisting of antimitotic, antikinase, alkylating, antimetabolite, antibiotic, alkaloid, anti-angiogenic, pro-apoptotic agents and combinations thereof. Other examples include paclitaxel, docetaxel, doxorubicin, cis platinum, and ricin.

[0129] In the following description, for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the present invention. It will be apparent, however, to one skilled in the art that the present invention may be practiced without these specific details. In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

6. Examples

[0130] The invention is illustrated herein by the experiments described by the following examples, which should not be construed as limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference. Those skilled in the art will understand that this invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will fully convey the invention to those skilled in the art. Many modifications and other embodiments of the invention will come to mind in one skilled in the art to which this invention pertains having the benefit of the teachings presented in the foregoing description. Although specific terms are employed, they are used as in the art unless otherwise indicated.

Example 1. Production of Anti-HDGF Antibody Secreting Hybridoma Clones C1, C4, H3, and L5-9.

[0131] To explore a bi-functional antibody-based therapeutic strategy, a panel of anti-HDGF antibodies (HDGF-C1, -C4, -H3, and L5-9) as previously described in Table 1 were created to bind native HDGF. In some embodiments, the antibodies are IgG1 and recognize HDGF and can recognize modified forms or variants, or fragments of HDGF. The cDNA fragment that encodes HDGF was PCR amplified and cloned into pGEX-4-T1 vector (GE Health Care, Piscataway, NJ). The resulting plasmid, pGST-HDGF, was used to generate GST-HDGF fusion protein in E. coli strain BL21 (DE) 3. The recombinant protein was purified using GST affinity chromatography. Balb/c mice were then immunized with the fusion protein and

boosted twice. Three days after the last boost, mice were sacrificed and spleenocytes were fused with P3X63Ag8.653 cells followed by culturing in selecting medium. Anti-HDGF antibody secreting hybridoma clones were identified and verified. For large scale antibody production, hybridoma cells were cultured in RPMI 1640 supplemented with Nutridoma CS (Roche Applied Science, Indianapolis, IN). The antibodies were purified using protein G-agarose (GE Health Care) affinity chromatograph. Purified antibody was then dialyzed and sterile filtered through a 0.22 μm filter. Sequence analysis produced the following amino acid sequences of antibody clones C1, C4, H3, and L5-9 set forth below in Table 2.

Table 2. Amino Acid Sequences of Antibody Clones C1, C4, H3, and L5-9

	Variable domains of anti-HDGF antibody clones H3, C1, C4, and L5
2.1 Clone H3	
2.1.1 H3HD4 (H3 heavy chain)	MGRLTSSFLLLVPPIYVLSQITLKQSGPGIVQPSQPVRLTCTFSGFSLSSTSGI GVAWIRQPSGKGLEWLATIWDDDNRYNPSLKSRLAVSKDTSNNQAFL NIITVETADTAIYYCAQIYDYAVGFAYWGQGLTVTVSAAKTPPSVYPLA PGSLGRAN (SEQ ID NO:9)
2.1.2 H3LF2 (H3 light chain)	MVSTAQFLGLLLLCFQGTRCDIQMIQTSSLSASLGDRVTISCRASQDISN YLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEPDD IATYYCQQYGKLWTFGGGTKEIKRADAAPTVSIFPPSSKLGKGEF (SEQ ID NO:10)
2.2 Clone C1	
2.2.1 C1HA5 (C1 heavy chain)	MNLGLSWIFFAVFYQG VHCVEQLVESGGRLVQPKGSLKLSAASGFTFN TYAMYWIRQAPGKGLEWVARIRSKSYNYATYYADSVKDRFTISRDDSQS MSYLQMNNLKTADTAMYYCVSEGFWGQTSVTVSSAKTPPSVYPLVP GSLGRANSADIHTG (SEQ ID NO:11)
2.2.2 C1LG1 (C1 light chain)	MKLPVRLLVLMFWIPASSSDVLMQTPLSLPVS LGDQASISCRSSQSIVHS SGNTYLEWYLQKPGQSPKLLIYKVS NRFSGV PDRFSGSGSGTDFTLKISR V EAEDLG VYYCFQGSHPWTFGGGTKEIKRADAAPTVSIFPPSSKLGKGE F (SEQ ID NO:12)
2.3 Clone C4	
2.3.1 C4HD1 (C4 heavy chain)	MDRLTSSFLLLIVPAYVLSQVTLKESGPGILQPSQTLSTCSFSGFSLSSTSG MGVGWIRQPSGKGLEWLAHIWDDVKRFNPDLKSRLTISKDTSSAQVFL KIASVDTADTATYYCTRIEDYDGALDYWGQTSVTVSSAKTPPSVYPLA PGSLGRAN (SEQ ID NO:13)
2.3.2 C4LA1 (C4 light chain)	MESQSQVFLSLLLWVSGTCGNIMMTQSPSSLA VSTGEMVTMSCKSSQSV LYSSNQKNYLAWFQQTPGQSPKLLIYWASTRESGV PDRFTGSGSGTDFTL TISNVQAADLAVYYCHQYLSSWTFGGGTKEIKRADAAPTVSIFPPSSKLG KGEF (SEQ ID NO:14)
2.4 clone L5 (also marked as L5-9)	
2.4.1 L5HB-1 (L5 heavy chain)	MKWSWVFLFLVATATGVR SQVQLQPGAELVKPGASVKLSCKASGYTF TSYWIHWVKQRPGQGLEWIGNINPNSGSTDNNEKFTSKATLTVDTSSTA YMQLSLTSEDSAVYYCTLLGRTGFAYWGQGLTVTVSAAKTPPSVYP LAPGSLGRAN (SEQ ID NO:15)
2.4.2 L5LD1 (L5 light chain)	MRAPAQILGILLWFPGIKCDIKMTQSPSSMYASLGERVTITCKASQDINS YLSWFQQKPGKSPKTLIYRANRLLDGVPSRFSGSGSGQDYSLTISSEYED LGIYYCLQYDEFPLTFGAGTKLELKRADAAPTVSIFPPSSKLGKGEF (SEQ ID NO:16)

Example 2. Humanized anti-HDGF H3 antibody (hH3)

[0132] The humanized anti-HDGF H3 (hH3) was created by grafting the complement determinant region (CDR) of mouse H3 to a human IgG1 framework. Methods for making monoclonal mouse H3 antibody are described in Ren, H., et al., “Antibodies targeting hepatoma-derived growth factor as a novel strategy in treating lung cancer,” Mol Cancer Ther. 2009 May; 8 (5): 1105-1112.

[0133] Humanization of H3 was performed to generate a humanized H3 (hH3) monoclonal antibody by the CDR grafting method. Usually, rodent antibodies can be immunogenic to

human and cause very serious side effects including the HAMA (human anti-mouse antibodies) response or anaphylactic shock. With this CDR grafting approach, CDR loops that make up the antigen-binding site of the mouse Mab are grafted into corresponding human framework regions. Initially, the variable light and heavy chain sequences of H3 were determined as shown in Table 2. To do so, H3 hybridoma cells were harvested by centrifugation and total RNA was extracted from cells. Total RNA was used for cDNA synthesis, and V-region genes of H3 were amplified, cloned and sequenced using standard primer sets.

[0134] CDR sequences were grafted into these VL and VH, such that the synthesized sequences each CDRs in the selected human framework sequences. To construct humanized H3 IgG1 in a mammalian expression vector, pLVBHN vectors were used. The following is a brief vector map. The vector was synthesized from several sources (e.g., see FIG. 9). However, it can be expressed from any mammalian expression vectors. The backbone of the vector for expression of recombinant antibody is modified from pTRIPZ (Open biosystems or GE Dharmacon), including LTR, psi, RRE, WPRE; the CMV promoter is from pHTN (Promega). However, any mammalian expression vector can be used to express the recombinant antibody. Transient transfection was used using Expi293 system from Invitrogen. Sequences are provided below; the CDRs are highlighted in bold.

2.1 Humanized H3 heavy chain variable region, mature sequence. SEQ ID NO:17.

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGIGVAWIRQPPGKALEWLATIWWD
DDNRYNPSLKSRLTITKDTSKNQVVLMTNMDPVDATYYCAQIYDYAVGFAYWGQG
TLVTVSS

2.2 Humanized H3 light chain variable region, mature sequence. SEQ ID NO:18.

DIQMTQSPSSLSASLGDRVTISCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLHSGV
PSRFGSGSGTDYSLTISSLPEDIATYYCQQYGKLWTFGQGTKLE

2.3 Humanized H3 heavy chain, mature sequence. SEQ ID NO:19.

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGIGVAWIRQPPGKALEWLATIWWD
DDNRYNPSLKSRLTITKDTSKNQVVLMTNMDPVDATYYCAQIYDYAVGFAYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TTPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC
PPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

2.4 Humanized H3 light chain, mature sequence. SEQ ID NO:20.

DIQMTQSPSSLSASLGDRVTISCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLHSGV
PSRFGSGSGTDYSLTISSLPEDIATYYCQQYGKLWTFGGGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Example 3: Construction of Chimeric H3 Antibody (cH3)

[0135] The chimeric H3 was constructed by fusing the variable domain of mouse anti-HDGF H3 with human IgG1 constant region. Sequences are provided below; the human IgG1 constant regions are highlighted in bold.

Table 3. Chimeric H3 sequences with variable domain from mouse and human IgG1 constant region.

3.1 Heavy chain. SEQ ID NO:21.

MVSTAQFLGLLLLCFQGTRCQITLKQSGPGIVQPSQPVRLTCTFSGFSLSTSGIGVAWI
RQPSGKGLEWLATIWDDDNRYNPSLKSRLAVSKDTSNNQAFNITVETADTAIYY
CAQIYDYAVGFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN
HYTQKSLSLSPGK

3.2 Light chain. SEQ ID NO:22.

MVSTAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFGSGSGTDYSLTISNLEPDDIATYYCQQYGKLW
TFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC

Example 4: Construction of Chimeric Bifunctional Antibodies H3K and H3T

Method of generating anti-HDGF hybridomas

[0136] Balb/c mice were immunized with recombinant HDGF in Freund's adjuvant.

Splenocytes from immunized animals were fused with P3x63Ag8.653 cells and screened for HDGF reactivity in the culture supernatant. Positive clones were identified and anti-HDGF

antibody secretion was verified by immunoblot analysis of cancer cell lysate and purified HDGF.

Determination of the amino acid sequences of anti-HDGF antibody

[0137] RNA from anti-HDGF antibody producing hybridoma was extracted, reverse transcribed into cDNA. Primers designed to amplify the Ig variable region (Larrick, J.W., et al. 1989. Biochem. Biophys. Res. Comm. 160, 1250. Jones, S.T. and Bendig, M.M. 1991. Biotechnology 9, 88.) were used to amplify the cDNA encoding the mouse Ig heavy and light chain. The amplified product were cloned and sequenced. The cDNA sequence that encoded the murine Ig heavy chain and light chain, and the CDRs were identified as described in <http://www.bioinf.org.uk/abs/>.

Generation of the chimeric antibody

[0138] The DNA fragments that encoded the murine antibody variable domain (VH and VL) were grafted to the N-terminal of the constant region of their human IgG1 constant part by standard molecular biology techniques, and cloned into a mammalian expression vector respectively. To produce the chimeric antibody, plasmids encoding for the heavy and light chain were co-transfected into Expi293 cells (Invitrogen) at a ratio of 1:2. The antibody was purified from the conditioned media by protein G affinity chromatograph.

Humanization of the murine anti-HDGF antibody H3

[0139] The cDNA sequence that encoded the murine Ig heavy chain and light chain, and the CDRs were identified as described by Martin A.C.R. (<http://www.bioinf.org.uk/abs/>). The humanized anti-HDGF Vh sequences were then created by replacing the CDRs in human Ig G1 Vh with the corresponding murine CDRs. The DNA sequence that encode the humanized sequence is then synthesized and grafted on the N-termini of the human IgG1 heavy chain constant region, cloned into a mammalian expression vector to generate a humanized Ig heavy chain expression construct. The humanized VL expression construct was created similarly. To produce the humanized anti-HDGF antibody, the plasmids that encode for the heavy and light chain was used to transfect Expi293 cell at a ratio of 1:2. The antibody was purified from the conditioned media by protein G affinity chromatograph.

Construction of Fc-fused growth factor binding domain (GF trapper)

[0140] Construction of Fc-VEGF trapper: DNA fragment corresponding to domain 2 and 3 (D2 and D3) of human VEGF receptor 2 (kinase insert domain receptor) amino acid residue 122 to residue 327 (based on NP_002253) was amplified from HEK 293 cDNA. The amplified sequence was fused to the C-terminus of the human IgG1 heavy chain via a poly-

GlySer linker, (GlyGlyGlyGlySer)₂, or (G4S)₂. The full-length anti-HDGF antibody heavy chain-VEGF trapper sequence was cloned into a mammalian expression plasmid.

[0141] Construction of Fc-TGFB trapper: DNA fragment corresponding to human TGFBR2 extracellular domain amino acid residue 27 to residue 184 (based on NP_001020018) was amplified from human lung cDNA. The amplified sequence was fused to the C-terminus of the human IgG1 heavy chain via a poly-GlySer linker, (GlyGlyGlyGlySer)₂, or (G4S)₂. The full-length anti-HDGF antibody heavy chain-TGFB trapper sequence was cloned into a mammalian expression plasmid.

Production of recombinant antibody (chimeric, and trapper)

[0142] To produce the recombinant anti-HDGF antibody, plasmids encoding for the heavy and light chain were co-transfected into Expi293 cells (Invitrogen) at a ratio of 1:2. The antibody was purified from the conditioned media by protein G affinity chromatograph.

Example 5. Treatment of MDA-2131-8 PDX Cells with H3K chimeric bifunctional antibody.

[0143] Human patient derived tumor xenograph (PDX) model 2131-8, a poorly differentiated adenocarcinoma of the lung, was implanted on the flank of athymic nude mice. As shown in FIG. 3, established tumor (150-400 mm³, 10 tumors per group) were treated with chemotherapy or combined chemo- plus antibody regimen. Arm A: PBS; Arm B: Gemcitabine, 1.2mg/kg; Arm C: Gemcitabine 1.2 mg/kg plus anti-HDGF H3 at 12.5mg/kg; Arm D: Gemcitabine 1.2 mg/kg plus anti-HDGF H3/VEGF trapper (H3K) at 12.5mg/kg. The drugs were given intraperitoneally every 3 days till tumor regression or sacrifice due to excess tumor burden. As shown in FIG. 3, the H3K bifunctional antibody induces extended tumor remission. Treatment was terminated after complete tumor remission in animals receiving Gemzar plus antibody. Therapy relapse were monitored. Arm C: Gemzar plus anti-HDFG H3 treated; Arm D: Gemzar plus anti-HDGF/VEGF trapper (H3K) treated. FIG. 4 shown extended remission in anti-HDGF plus chemo treatment, and even fewer relapse in h3k plus chemo group.

Example 6. Treatment of bleomycin induced lung fibrosis.

[0144] FIG. 5 illustrates results for mouse survival after bleomycin (36ug/animal) was given intratracheally to C57/B6 mice (5 – 6 weeks old, 7 per group) under anesthesia. Antibodies were given twenty-four hours later intraperitoneally in the following treatment groups: 1) mouse anti-HDGF H3 plus anti-HDGF C1 125ug each (H3+C1); 2) anti-HDGF H3 250ug plus Avastin 100 µg (H3+A); 3) PBS. The treatments were given every 3 days thereafter for a total 6 doses. Animals were monitored daily. For the data provided in FIG. 6, mice (5 – 6 weeks old, 7 per group) were primed with antibody intraperitoneally according to the

following treatment groups: 1) anti-HDGF H3 plus anti-HDGF C1 125ug each (H3+C1); 2) chimeric anti-HDGF H3/VEGF trapper plus anti-HDGF C1, 125ug each (H3K + C1); 3) chimeric anti-HDGF H3/TGFB trapper plus anti-HDGF C1, 125ug each; 4) PBS control group (PBS). Bleomycin (36ug/animal) was given intratracheally 24-hours later under anesthesia. Antibodies were again given on day 3 (24-hr post-bleomycin instillation), and every three days thereafter for a total of 7 doses (including priming). Animals were monitored daily. These data indicate anti-HDGF antibody, give prior or post bleomycin instilment delayed the onset and reduced the mortality of animals due to lung fibrosis. FIG. 7 shows high-level, sustained HDGF expression after Bleomycin treatment in mouse lung. FIG. 8 shown histological changes in bleomycin treated animals. Hyper-proliferation of fibroblast in mouse lung after bleomycin treatment was seen in untreated or shame (PBS) treated animals (FIG. 8B and FIG. 8C), whereas anti-HDGF treatment reduce the severity of lung fibrosis.

Example 7. Human NSCLC PDX Tumor MDA274 Response to Anticancer Therapies.

[0145] Nude mice carrying human NSCLC PDX tumor MDA274 were subjected to anticancer therapies. Using the methods described in Example 5, above, a second different human patient derived tumor xenograph (PDX) model, also a poorly differentiated adenocarcinoma of the lung, was implanted on the flank of athymic nude mice. Drugs were administered every three days.

[0146] As shown in FIG. 11, established tumor (10 tumors per group) were treated with chemotherapy or with combined chemotherapy plus antibody regimen. Tumor volumes were measured every three days. FIG. 11A shows a summary of average tumor volumes after five treatment regimens. Diamonds indicate negative control (PBS) treatment; squares indicate chemotherapy (Gemcitabine, 50 mg/kg, and Pemetrexed, 30 mg/kg); triangles indicate chemotherapy as above plus anti-VEGF antibody, 12.5 mg/kg; X indicates chemotherapy as above plus anti-HDGF antibody, 12.5 mg/kg; asterisk indicates chemotherapy as above plus H3K (anti-HDGF/anti-VEGF bifunctional antibody), 12.5 mg/kg.

[0147] FIG. 11B and FIG. 11C show the tumor volumes of individual mice with treatment consisting of chemotherapy as above plus anti-VEGF antibody (FIG. 11B) and chemotherapy as above plus H3K anti-HDGF/anti-VEGF bifunctional antibody. Progression-free survival was routinely observed in the chemotherapy (Gemcitabine/Pemetrexed) plus H3K group (see FIG. 11C).

Example 8. Immunohistochemical Staining.

[0148] Mice bearing MDA 2131-8 (PDX) tumors were euthanized at 30% and 70% tumor volume reduction and the tumors dissected (about 500 mm³). Thus, tumor tissues at 30% and 70% volume reduction were collected.

[0149] The tissues were prepared as follows. The tissues were fixed in 4% formaldehyde-PBS, and embedded in paraffin. Four-micron (4 µm) sections of the embedded tissue were cut, mounted on Superfrost® plus microscope slides. The slides were baked at 55 °C, and deparaffined in xylene, followed by rehydration in graded alcohol solutions. The slides then were treated in 50 mM Tris-HCl, pH 8.5 for 30 minutes at 100 °C to retrieve antigen, quenched in 3% hydrogen peroxide and blocked in 1% goat serum. Primary antibody at the manufacture recommended dilution (1:100 to 1:1000) was added and incubated with the tissue section at 4 °C overnight. The following day, the sections were washed with PBS, then developed with VECTORSTAIN ABC® HRP kit or Vector MOM Elite® HRP kit per the manufacture's standard protocol, using diaminobenzidine (DAB) as the chromogenic agent. The developed sections were counterstained with hematoxylin, dehydrated in graded alcohol and xylene, and mounted in Permount® mounting medium. Results are presented in FIG. 12, FIG. 13, and FIG. 14 at magnification 20x.

[0150] For FIG. 12, MDA 2131-8 tissue was stained with anti-SOX2 antibody from Cell Signaling Technology® CST3579. FIG. 12A: treatment naïve tumor; FIG. 12B: Gemcitabine plus anti-VEGF treatment at 30% tumor volume reduction; FIG. 12C: Gemcitabine plus H3K bifunctional antibody treatment at 30% tumor volume reduction. These results show significant reduction of SOX2-positive tumor cells for samples treated with H3K compared to anti-VEGF treatment. SOX2 is a stem cell factor essential for maintaining cell renewal of undifferentiated embryonic stem cells. It is re-expressed in some cancer cells, and is correlated with more aggressive tumor phenotypes and drug resistance.

[0151] For FIG. 13, MDA 2131-8 tissue was stained with anti-CD34 antibody from Abcam® ab81289. FIG. 13A: treatment naïve tumor; FIG. 13B: Gemcitabine plus anti-VEGF treatment at 70% tumor volume reduction; FIG. 13C: Gemcitabine plus H3K bifunctional antibody treatment at 70% tumor volume reduction. CD34 is a marker of hematopoietic and mesenchymal stem cells, as well as endothelial progenitor cells. These results therefore show that in tumor treated with anti-VEGF, a significant amount of CD34-positive vasculature remains, whereas in H3K-treated tumor, no intact CD34-staining vasculature was found.

[0152] For FIG. 14, MDA 2131-8 tissue was stained with anti-CD31 antibody from Invitrogen® 170700. FIG. 14A: treatment naïve tumor; FIG. 14B: Gemcitabine plus anti-VEGF treatment at 70% tumor volume reduction; FIG. 14C: Gemcitabine plus H3K bifunctional antibody treatment at 70% tumor volume reduction. CD31 is a marker of endothelial cells. These results therefore show that in tumor treated with anti-VEGF, a significant amount of CD31-positive vasculature remains, whereas in H3K-treated tumor, no intact CD31-staining vasculature was found.

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[0153] All references cited herein are hereby incorporated by reference in their entirety.

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CLAIMS

What is claimed is:

1. A bifunctional hepatoma-derived growth factor (HDGF)-specific antibody comprising (1) at least one complementarity determining region (CDR) specific for HDGF, and (2) at least one receptor domain that specifically binds to a growth factor selected from vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF β).
2. The antibody of claim 1, wherein the CDR is selected from a mouse, human, rabbit, or rat CDR.
3. The antibody of claim 2, wherein the CDR is a mouse CDR.
4. The antibody of claim 1, which comprises two receptor domains each of which independently bind to a growth factor selected from VEGF and TGF β .
5. The antibody of claim 4, wherein the TGF β is selected from the group consisting of TGF β subtype 1, subtype 2, and subtype 3.
6. The antibody of claim 2, wherein the antibody is H3K or H3T.
7. The antibody of claim 2, wherein the antibody is humanized or chimeric.
8. The antibody of claim 2, wherein the antibody selectively binds both HDGF and VEGF or both HDGF and TGF β .
9. The antibody of claim 1, wherein the antibody is linked to a reporter molecule or an effector molecule.
10. The antibody of claim 9, wherein the reporter molecule is selected from the group consisting of an enzyme, a radiolabel, a hapten, a fluorescent label, a phosphorescent molecule, a chemiluminescent molecule, a chromophore, a luminescent molecule, a photoaffinity molecule, a ligand, a colored particle and biotin.
11. The antibody of claim 9, wherein the effector molecule is selected from the group consisting of a toxin, an apoptotic molecule, an antitumor agent, a therapeutic enzyme and a cytokine.
12. The antibody of claim 11, wherein the antitumor agent is selected from the group consisting of gemcitabine, pemetrexed, cisplatin, docetaxel, vinorelbine, doxorubicin, 6-fluorouracil, erlotinib, gefitinib, and crizotinib.
13. A pharmaceutical composition comprising the antibody of any one of claims 1-12 and a pharmaceutically acceptable carrier.
14. The pharmaceutical composition of claim 13 which further comprises a cancer chemotherapeutic drug.

15. The pharmaceutical composition of claim 14, wherein the cancer chemotherapeutic drug is gemcitabine.
16. The pharmaceutical composition of claim 13, which is formulated for parenteral, intravenous, local, or topical administration.
17. The pharmaceutical composition of claim 16, which is formulated for administration in an amount the antibody from 5 to 25 mg/kg every 1-3 weeks.
18. A method for reducing the growth of hyperproliferative cells in a subject comprising administering to said subject an amount of an antibody of any one of claims 1-12 that down-regulates HDGF and VEGF expression or HDGF and TGF β expression and is effective to reduce the growth of hyperproliferative cells.
19. The method of claim 16, wherein the hyperproliferative cell is a fibroblast, vascular endothelial cell, vascular smooth muscle cell or a cancer cell.
20. The method of claim 17, wherein the cancer cell is selected from the group consisting of lung cancer, pancreatic cancer, colon cancer, ovarian cancer, liver cancer, glioblastoma, and squamous cell carcinoma.
21. The method of any one of claims 18-20 which further comprises administering a cancer chemotherapeutic drug.
22. The method of claim 21, wherein the cancer chemotherapeutic drug is gemcitabine.
23. A method for simultaneously reducing expression of HDGF and VEGF or HDGF and TGF β in a patient in need thereof by administering an antibody of any one of claims 1-12.
24. The method of claim 23, wherein the patient suffers from a hyperproliferative condition selected from the group consisting of interstitial lung disease, keloids, lung fibrosis, idiopathic pulmonary fibrosis, pulmonary hypertension, diabetic retinopathy, age-related macular degeneration, lung cancer, pancreatic cancer, colon cancer, ovarian cancer, liver cancer, glioblastoma, and squamous cell carcinoma.
25. The method of any one of claims 23-24, which further comprises administering a cancer chemotherapeutic drug.
26. The method of claim 25, wherein the cancer chemotherapeutic drug is gemcitabine.

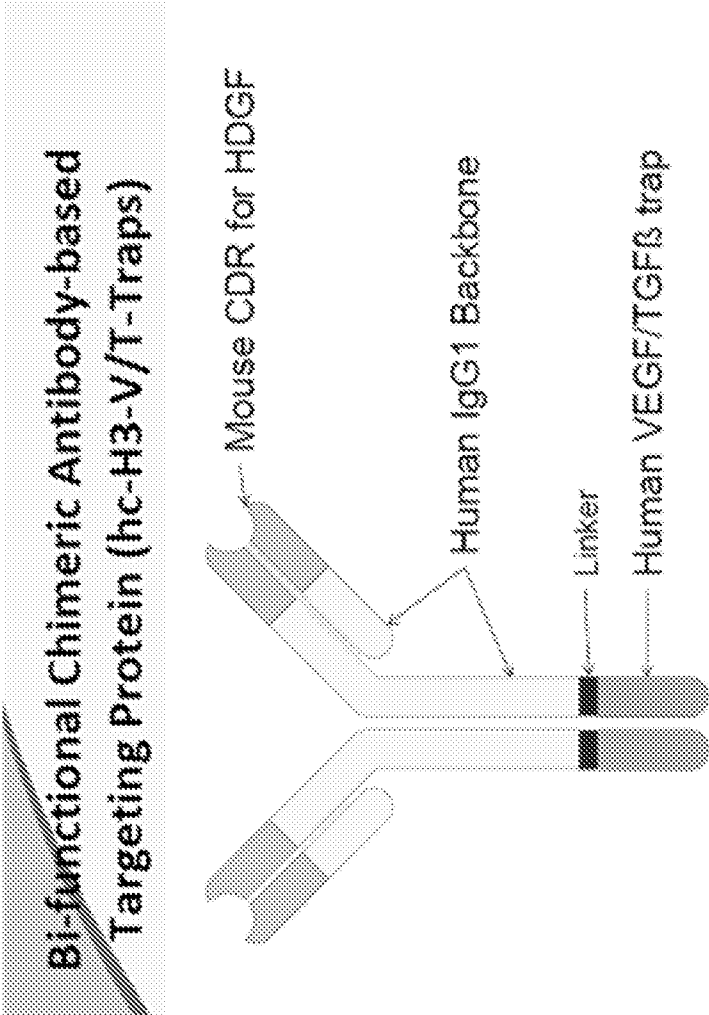


FIG. 1

Bi-functional Chimeric Antibody-based
Targeting Protein (hc-H3-V/T-Traps)

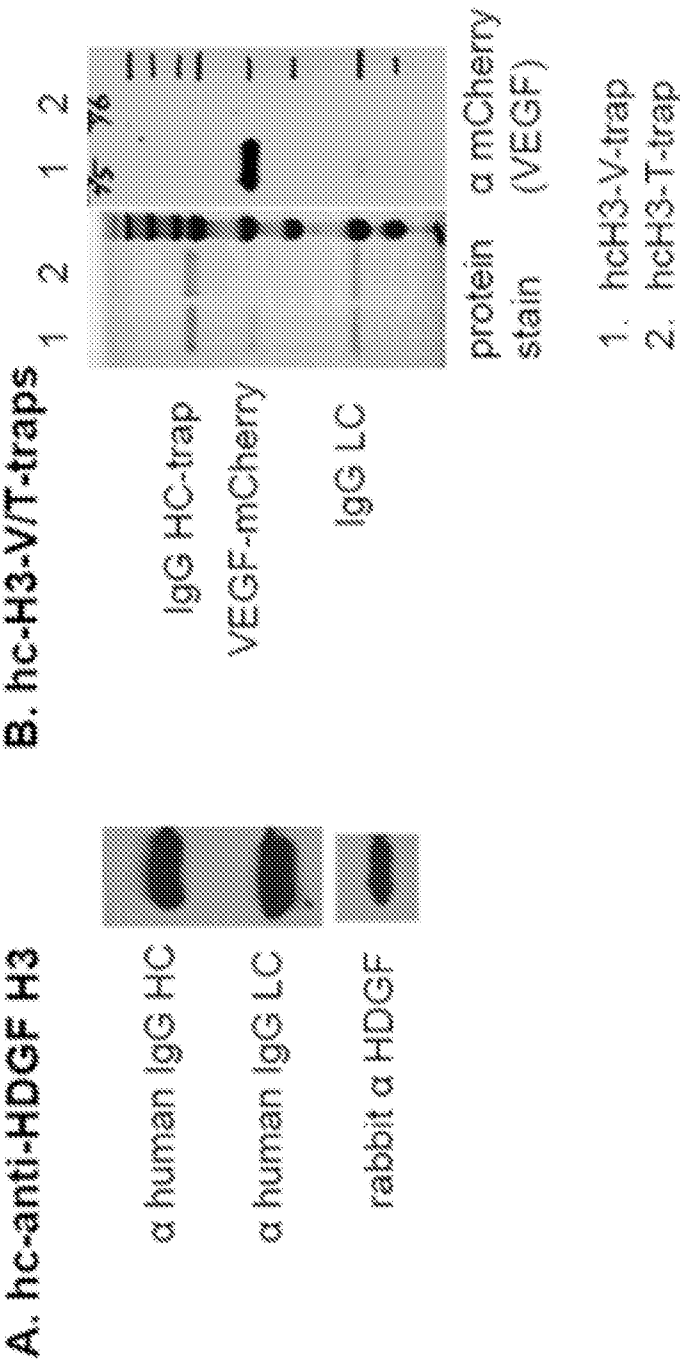


FIG. 2A

FIG. 2B

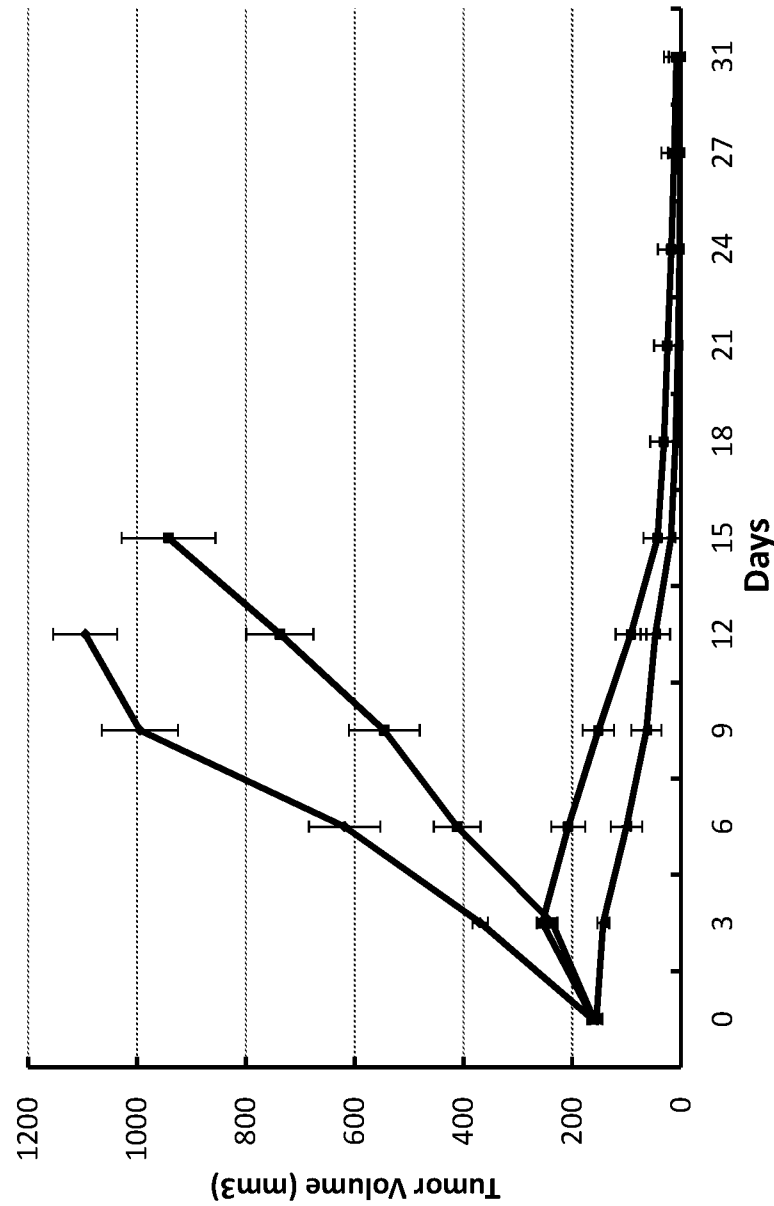


FIG. 3

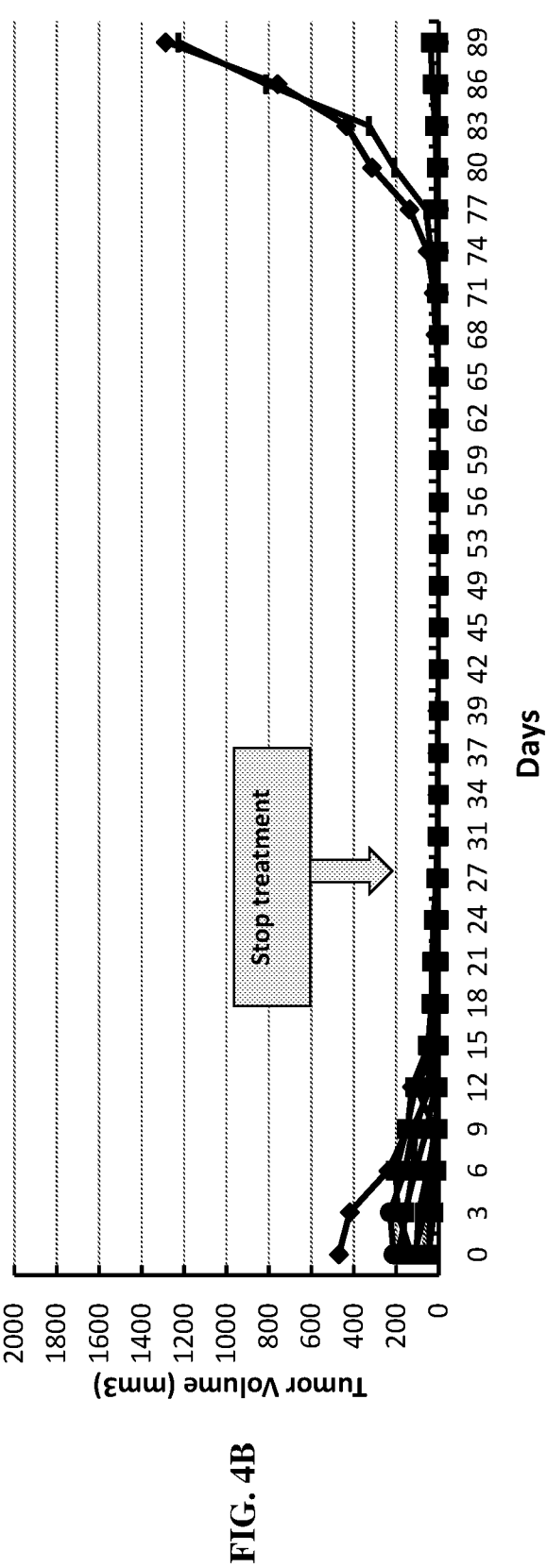
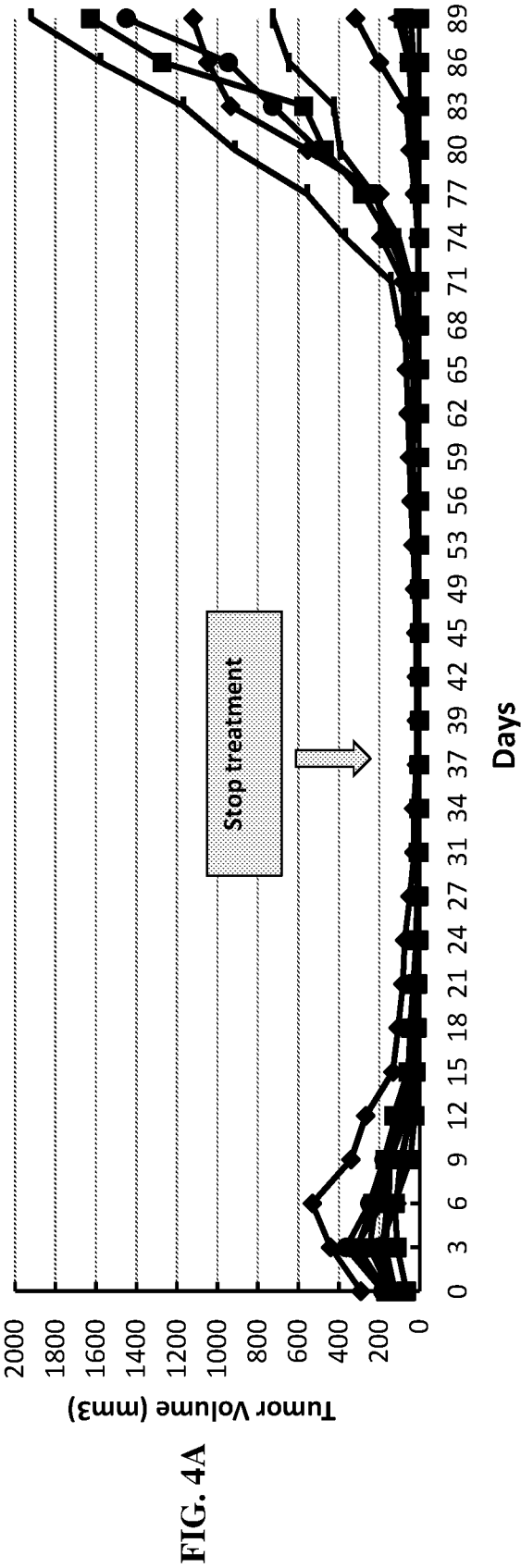


FIG. 5

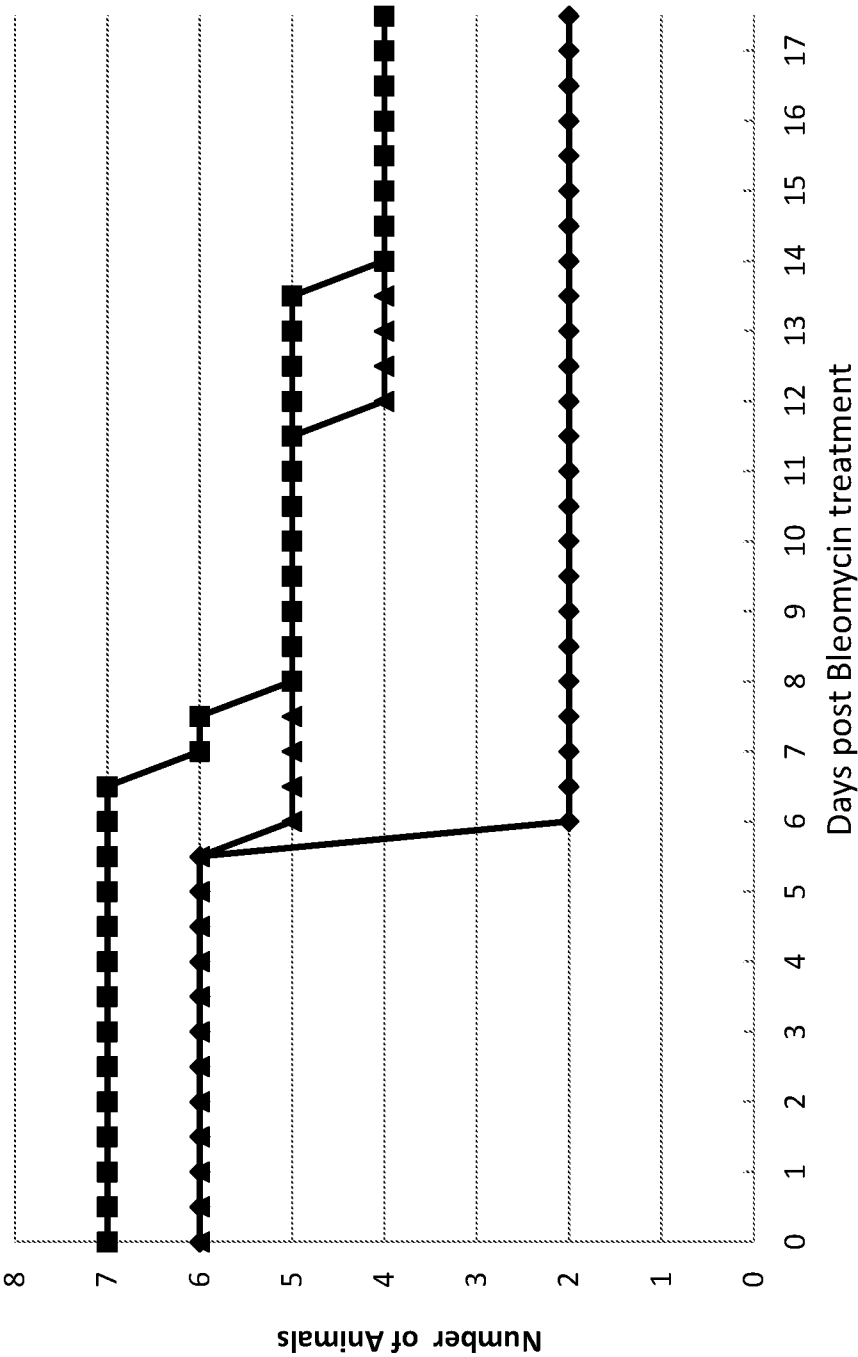


FIG. 6

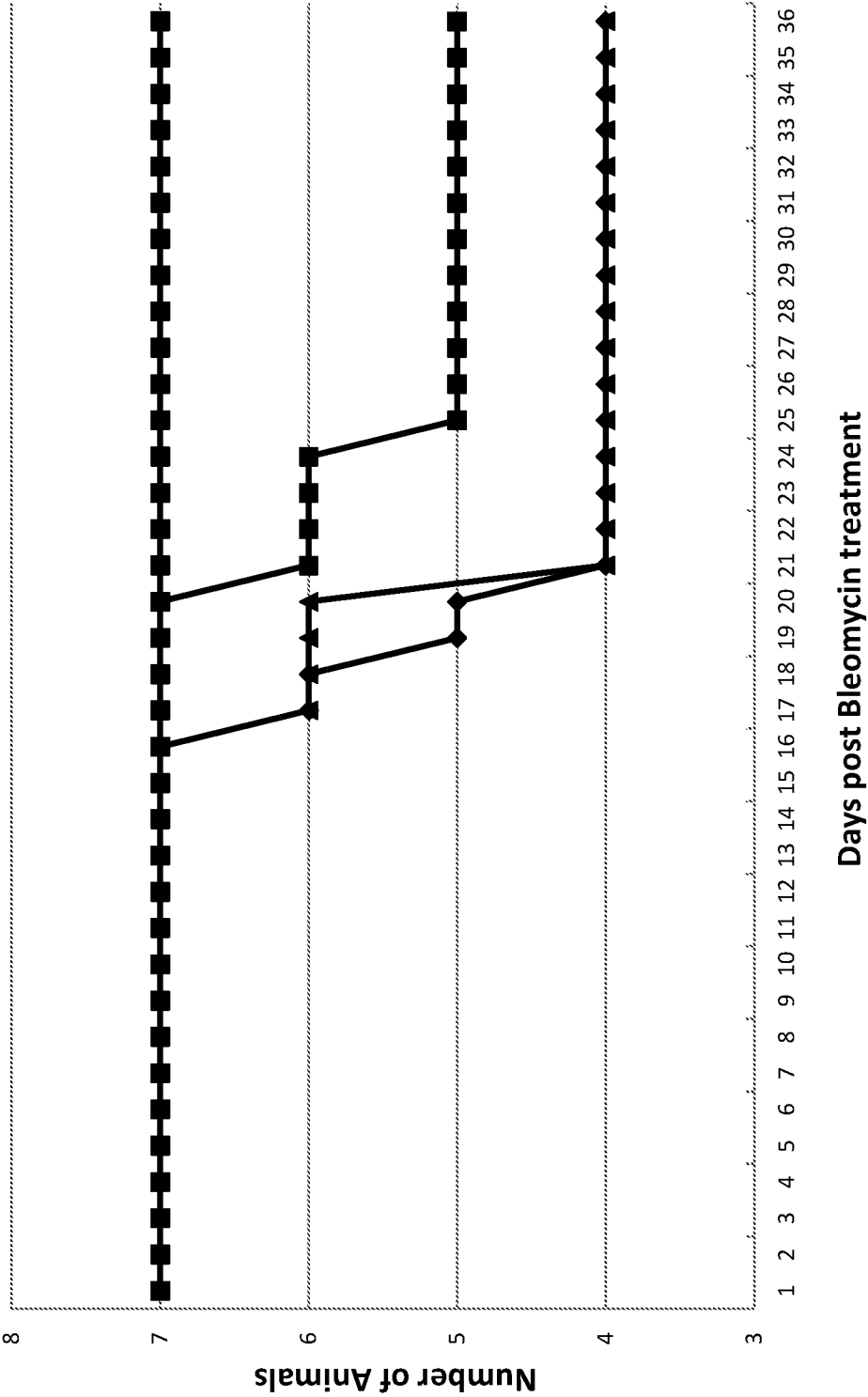


FIG. 7A

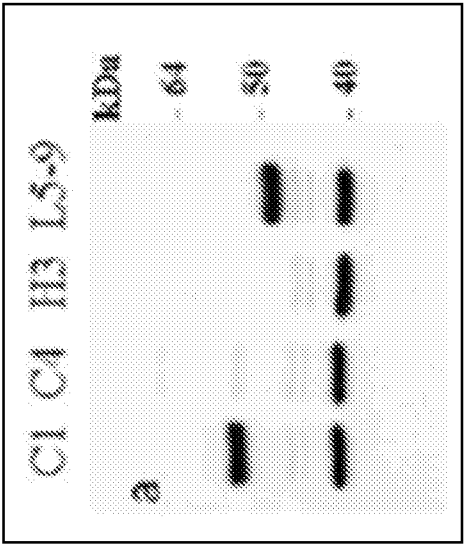
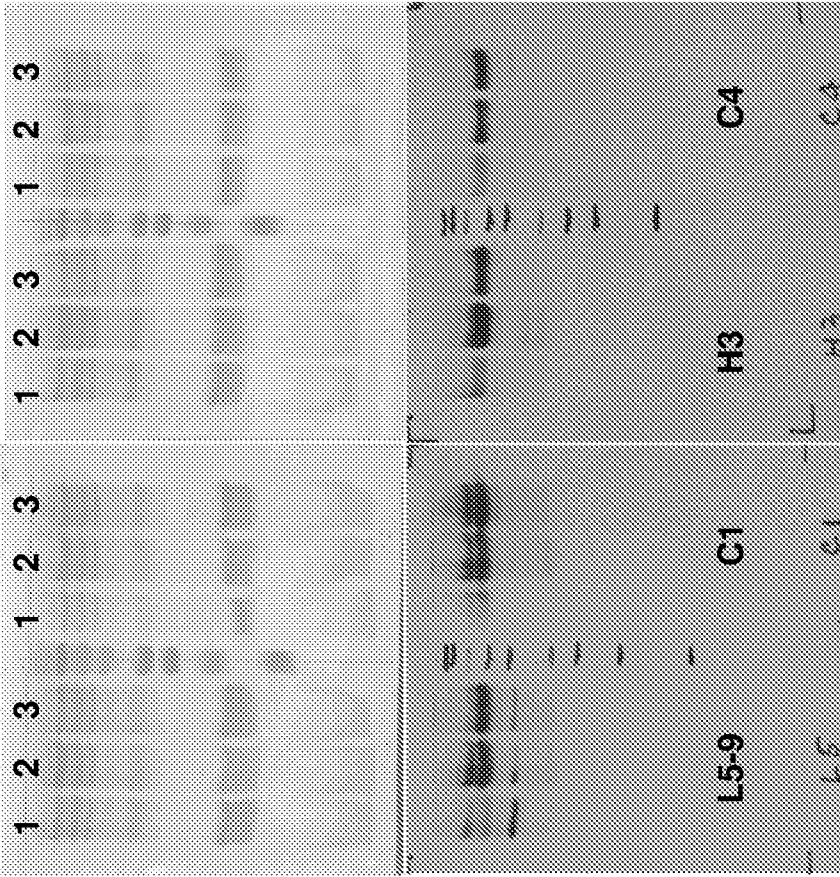


FIG. 7B



Reorganization of native and post-transcriptionally modified HDGF by various antibodies.

FIG. 8A



4x

FIG. 8B

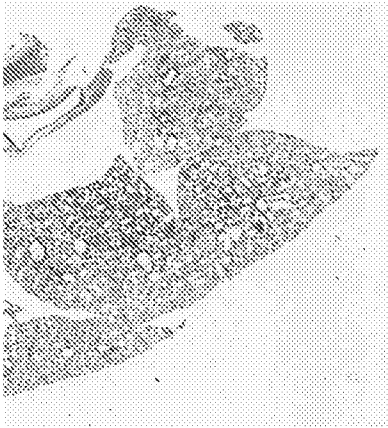


FIG. 8C

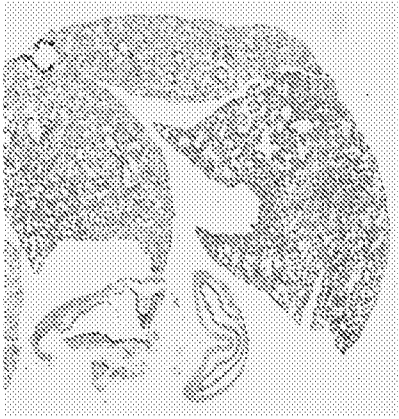
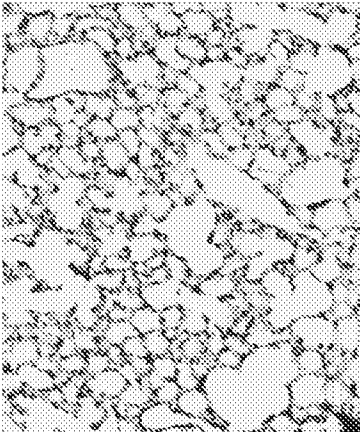
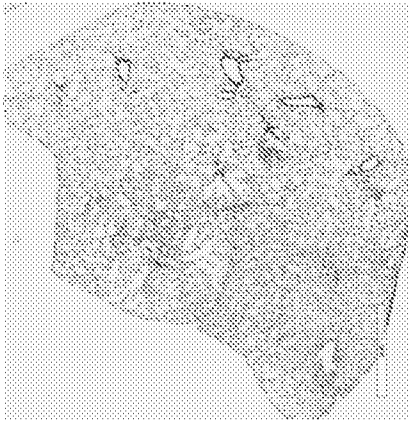
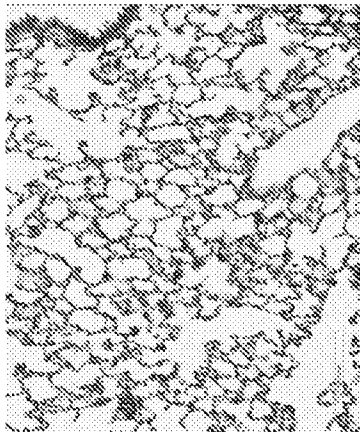
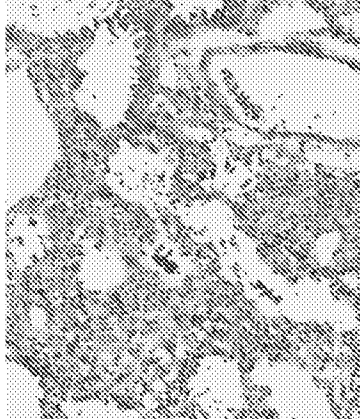
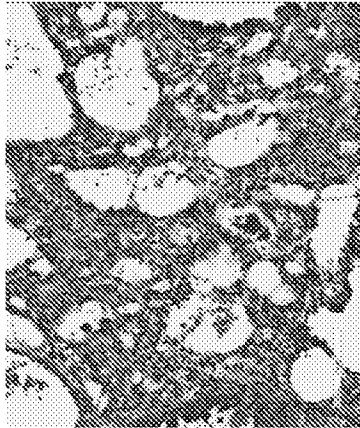


FIG. 8D



20x



Normal

Bleomycin

PBS

H3 + C1

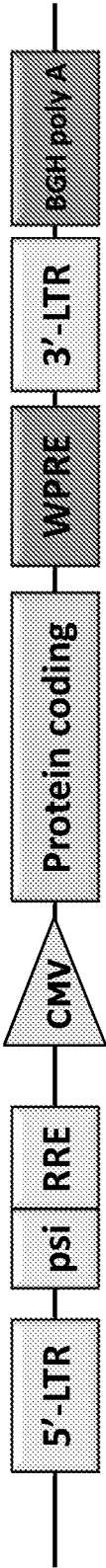
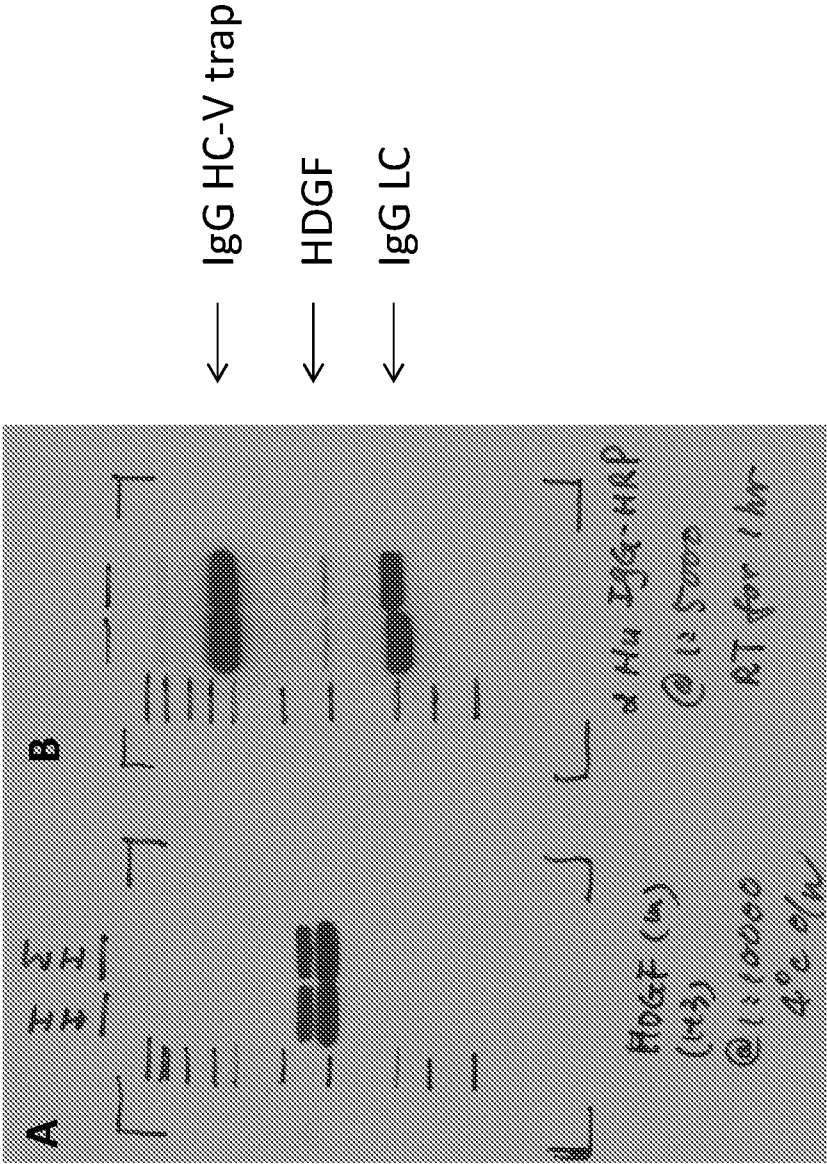


FIG. 9

FIG. 10A FIG. 10B



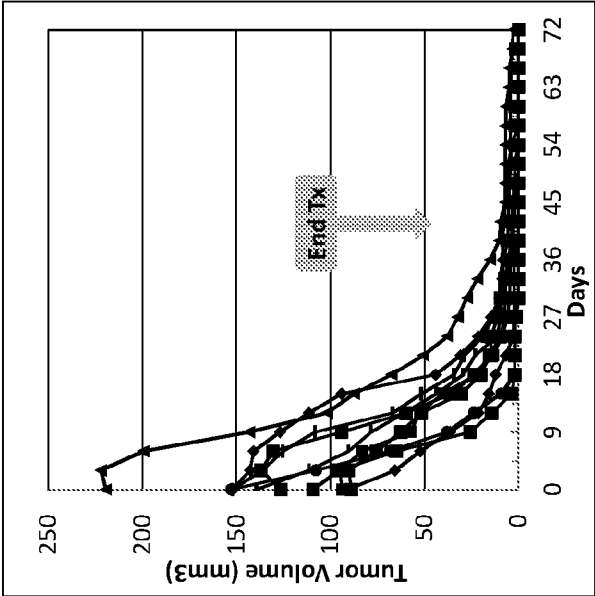


FIG. 11C

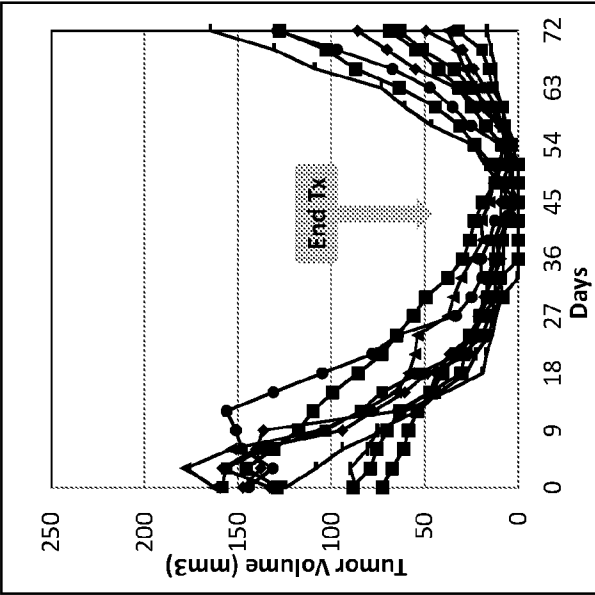


FIG. 11B

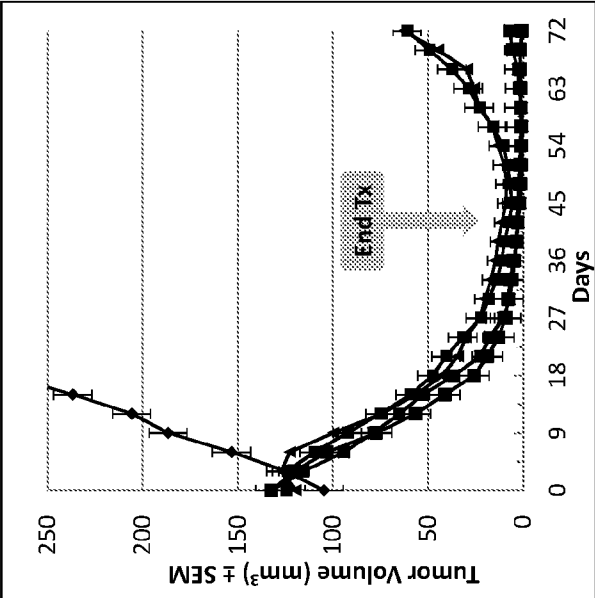


FIG. 11A

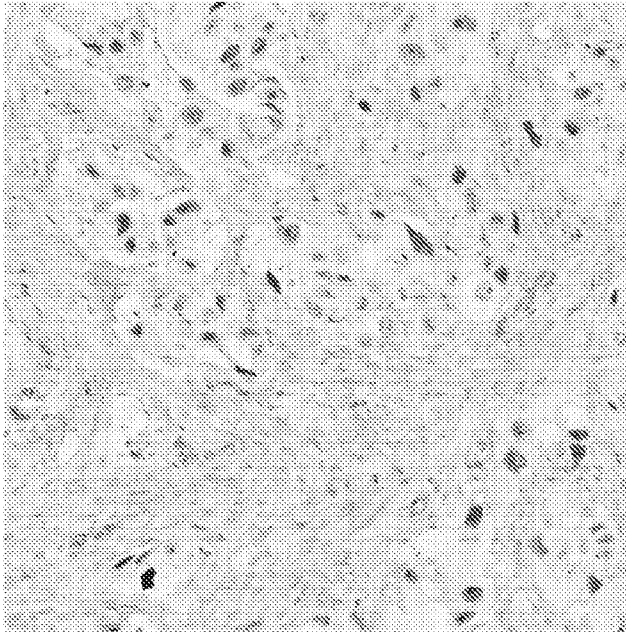


FIG. 12C

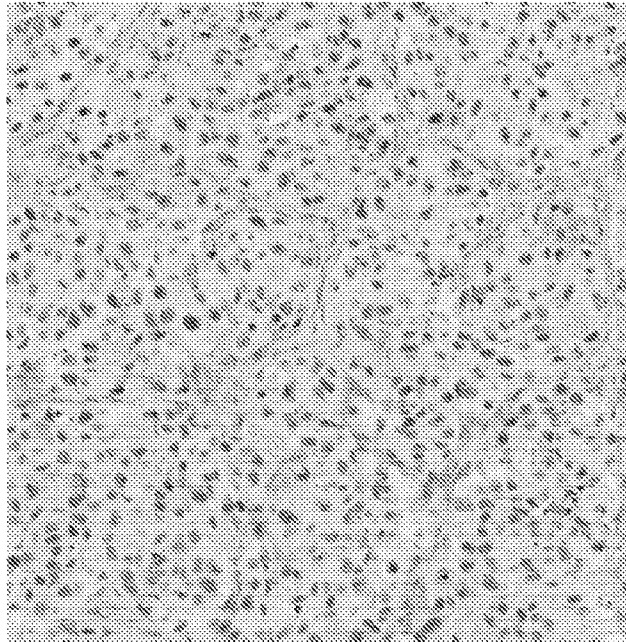


FIG. 12B

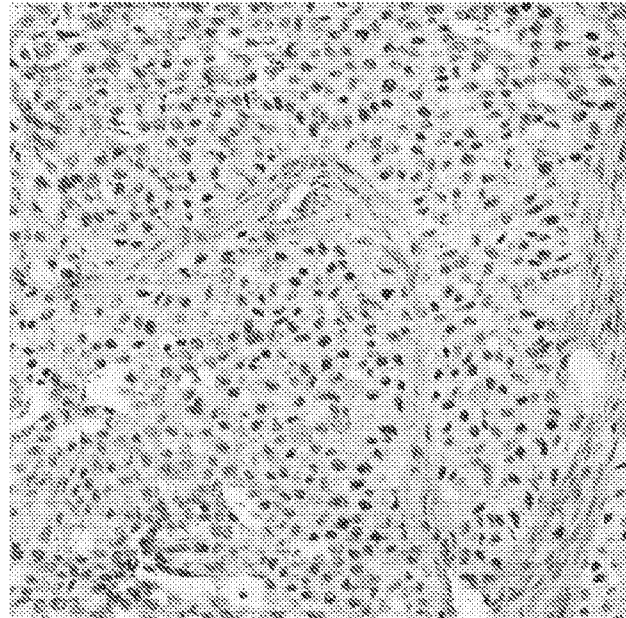


FIG. 12A

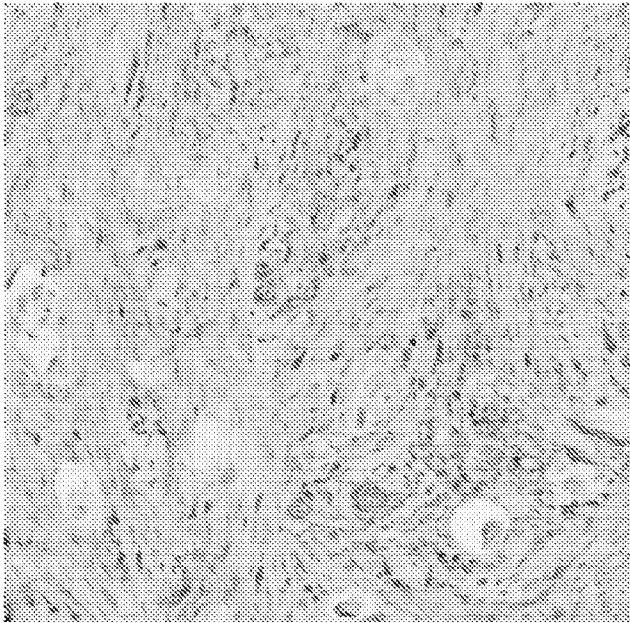


FIG. 13C

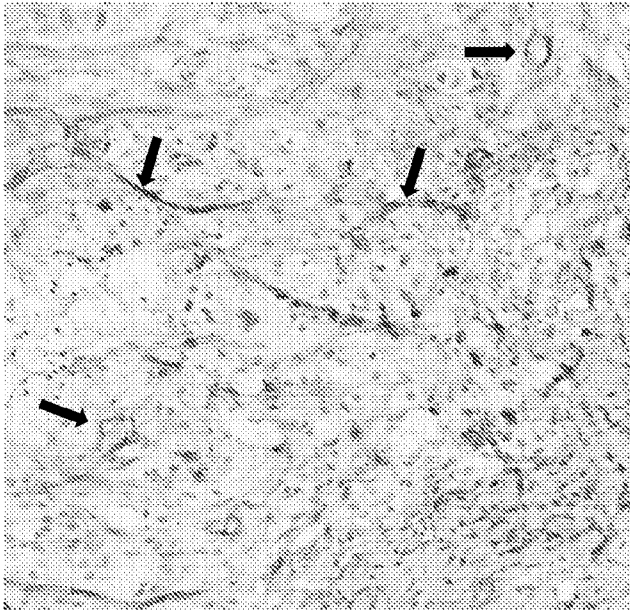


FIG. 13B

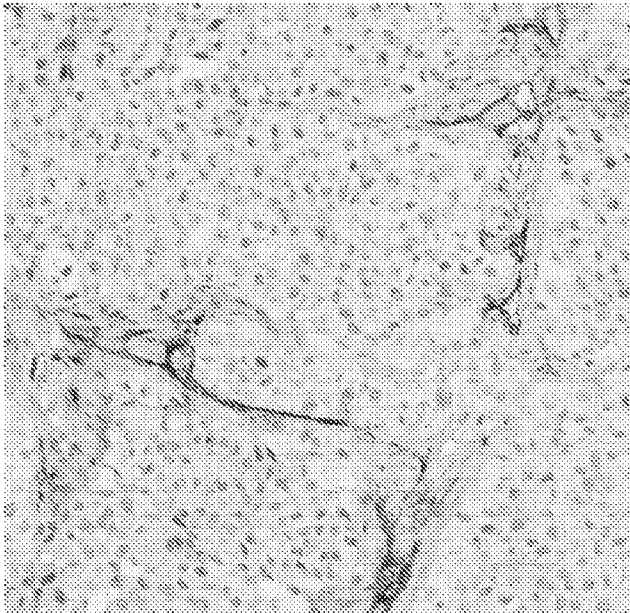


FIG. 13A

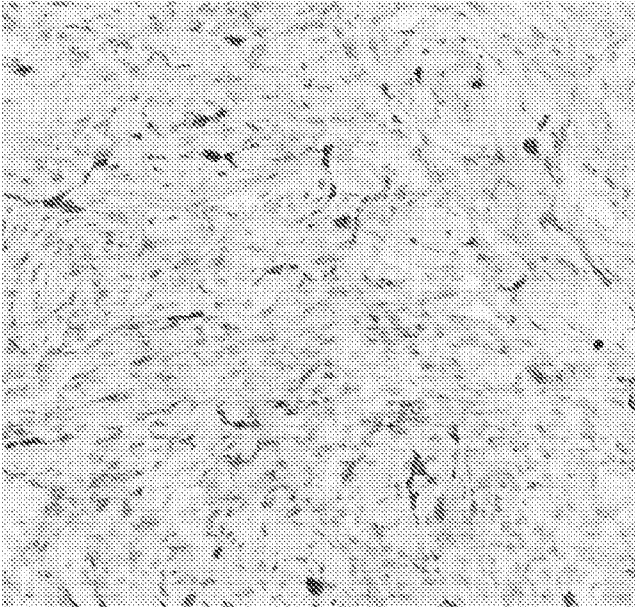


FIG. 14C

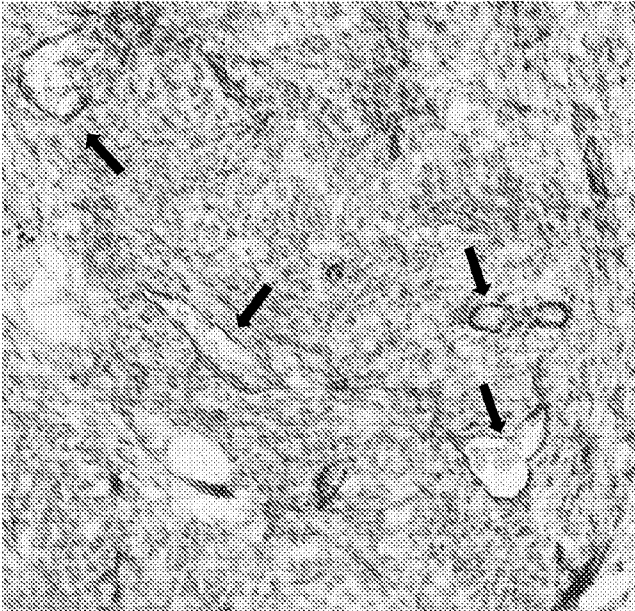


FIG. 14B

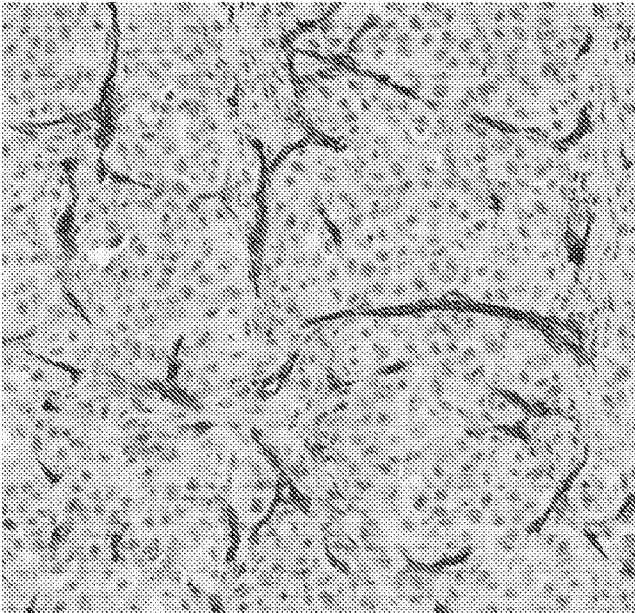


FIG. 14A

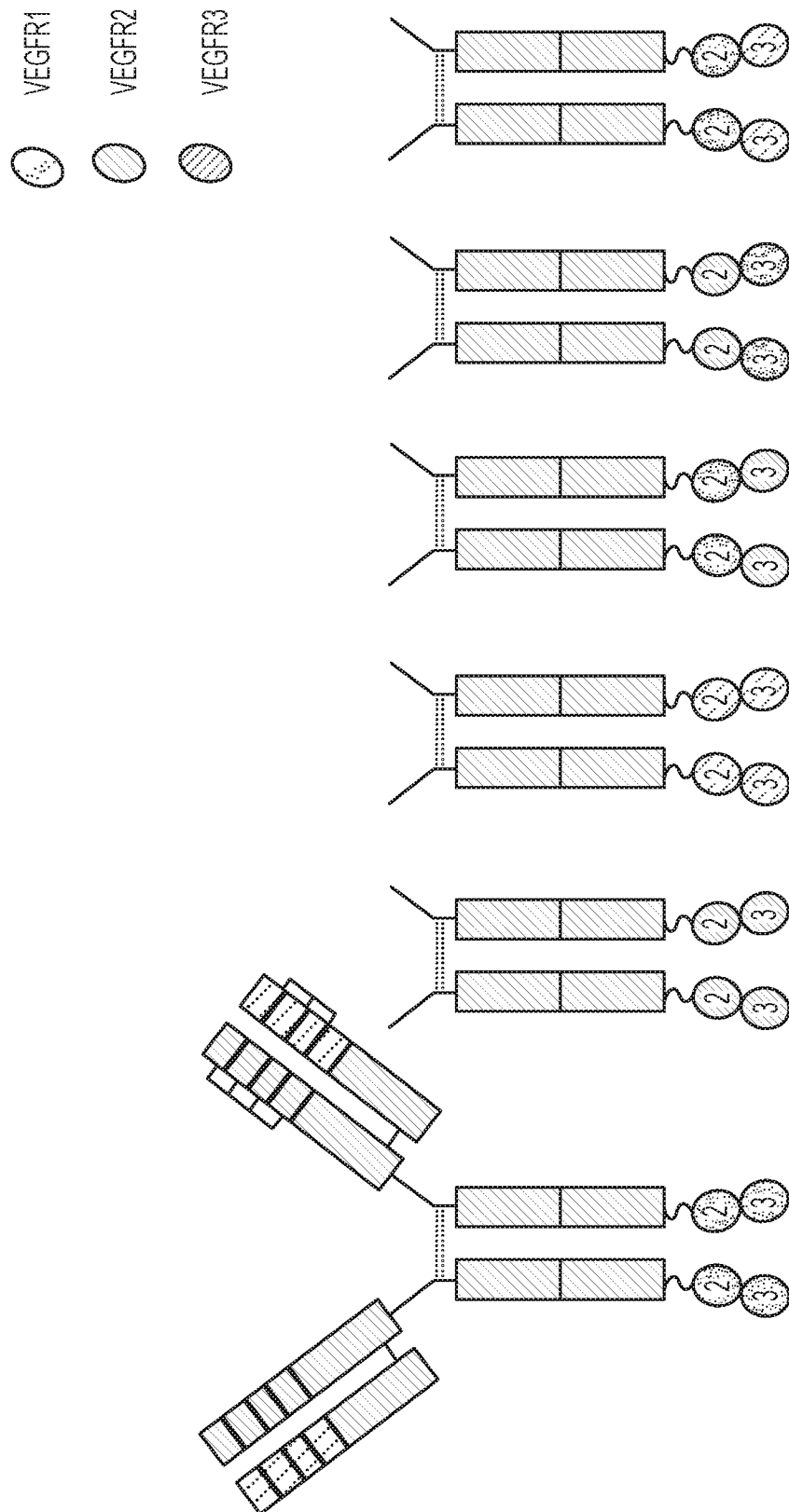


FIG. 15A

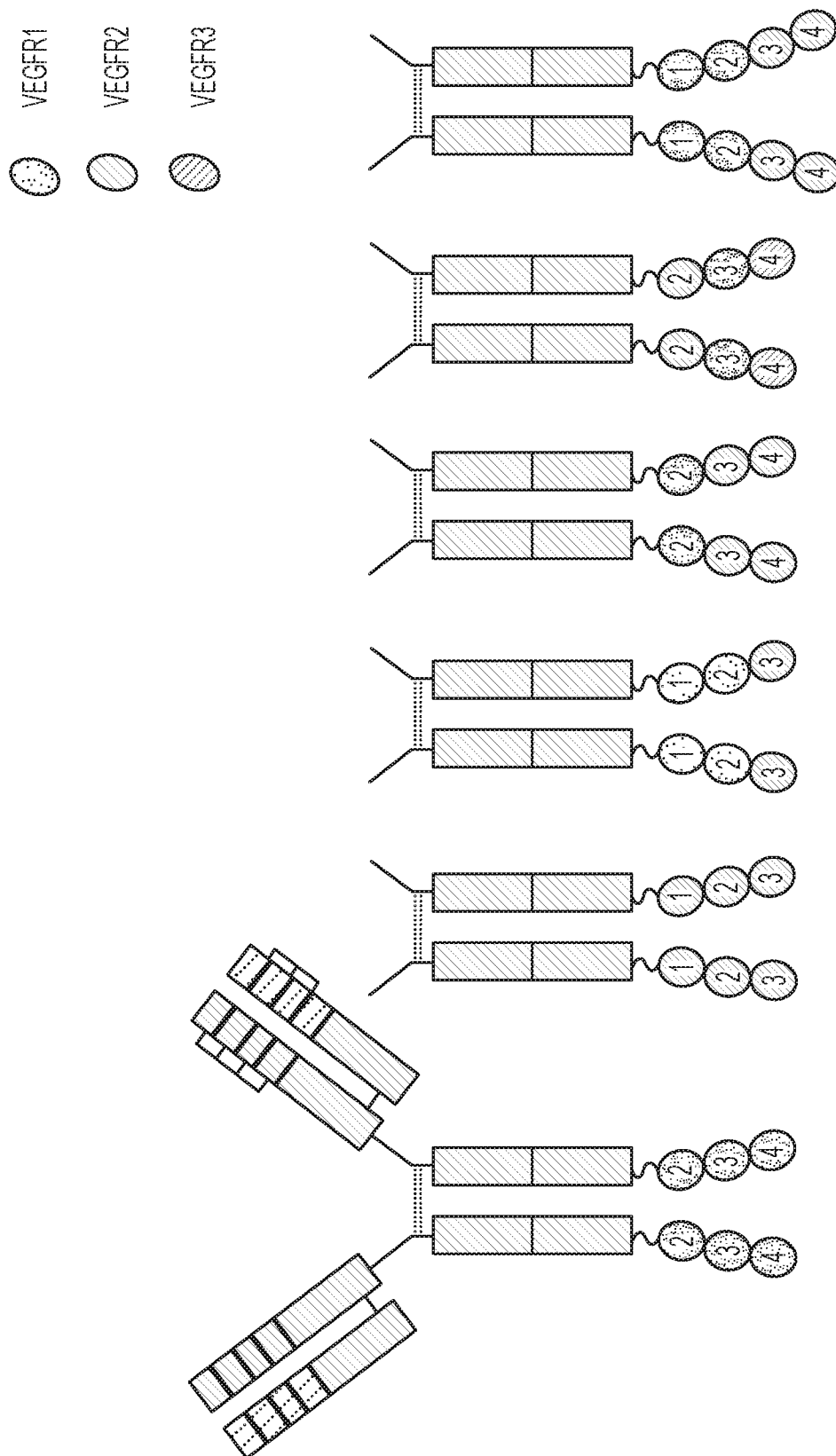


FIG. 15B