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(71) Demandeur/Applicant:  
AMGEN INC., US

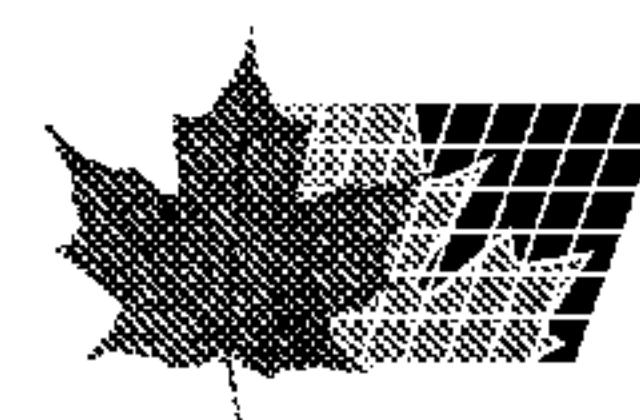
(72) Inventeur/Inventor:  
KE, HUA ZHU, US

(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : PROCEDE DE TRAITEMENT D'UNE FRACTURE OSSEUSE A L'AIDE D'ANTICORPS ANTI-SCLEROSTINE  
(54) Title: METHOD FOR TREATING BONE FRACTURE WITH ANTI-SCLEROSTIN ANTIBODIES

(57) Abrégé/Abstract:

The invention provides a method of enhancing bone fracture healing involving administering a sclerostin inhibitor. In one aspect, the invention includes use of a therapeutically effective amount of sclerostin binding agent to treat a bone fracture, wherein one or more administrations of the sclerostin binding agent are administered over a treatment period lasting at least two weeks and beginning within two weeks of the fracture.



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(71) Applicant (for all designated States except US): AMGEN INC. [US/US]; Patent Operations, M/S 28-2-C, One Amgen Center Drive, Thousand Oaks, CA 91320-1799 (US).

## (72) Inventor; and

(75) Inventor/Applicant (for US only): KE, Hua, Zhu [US/US]; 5258 Via Dolores, Newbury Park, CA 91320 (US).

(74) Agents: KISSLING, Heather, R. et al.; Marshall, Gerstein &amp; Borun LLP, 233 S. Wacker Drive, Suite 6300, Sears Tower, Chicago, IL 60606-6357 (US).

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## (54) Title: METHOD FOR TREATING BONE FRACTURE WITH ANTI-SCLEROSTIN ANTIBODIES

(57) Abstract: The invention provides a method of enhancing bone fracture healing involving administering a sclerostin inhibitor. In one aspect, the invention includes use of a therapeutically effective amount of sclerostin binding agent to treat a bone fracture, wherein one or more administrations of the sclerostin binding agent are administered over a treatment period lasting at least two weeks and beginning within two weeks of the fracture.

**METHOD FOR TREATING BONE FRACTURE WITH ANTI-SCLEROSTIN ANTIBODIES**

**[0001]** The invention generally relates to methods of using sclerostin inhibitors to enhance bone fracture healing.

**CROSS REFERENCE TO RELATED APPLICATIONS  
AND INCORPORATION BY REFERENCE**

**[0002]** This application claims priority to U.S. Provisional Patent Application No. 61/013,917, filed December 14, 2007. In addition, the following applications are hereby incorporated by reference in their entirety: U.S. Provisional Patent Application No. 60/973,024, filed September 17, 2007, U.S. Patent Application No. 11/410,540, filed April 25, 2006, which claims priority to U.S. Provisional Patent Application No. 60/792,645, filed April 17, 2006, U.S. Provisional Patent Application No. 60/782,244, filed March 13, 2006, U.S. Provisional Patent Application No. 60/776,847, filed February 24, 2006, and U.S. Provisional Patent Application No. 60/677,583, filed May 3, 2005; and U.S. Patent Application No. 11/411,003, filed April 25, 2006, which claims priority to U.S. Provisional Patent Application No. 60/792,645, filed April 17, 2006, U.S. Provisional Patent Application No. 60/782,244, filed March 13, 2006, and U.S. Provisional Patent Application No. 60/776,847, filed February 24, 2006.

**BACKGROUND OF THE INVENTION**

**[0003]** A bone fracture is a break or crack in bone that can result in pain, swelling, injury to soft tissue, and bruising from internal bleeding. Anyone, regardless of age, race, or economic background, is vulnerable to bone fractures. Fractures are most often caused by physical trauma, such as a vehicle accident, physical abuse, or serious fall. Low bone mineral content, however, drastically increases a person's susceptibility to fractures. For example, osteoporosis-related injury represents a significant medical challenge, particularly for the elderly for whom increased bone fragility is aggravated by a greater risk of accidental falls. Fractured hips, wrists, and vertebrae are among the most common injuries associated with osteoporosis. Hip fractures in particular are extremely uncomfortable and expensive for the patient and, for women, correlate with high rates of mortality and morbidity.

**[0004]** In most cases, fractures are treated by immobilization with a splint, cast, or brace, combined with limiting activity for an extended period of time. The impact of fracture treatment on the patient and patient's family is profound. Direct costs of fracture treatment often include hospital expenses and physical therapy. In 1995, medical expenses associated

with treatment of forearm fractures in the United States amounted to \$385 million (Ray et al., *J. Bone Miner. Res.*, 12(1):24-25 (1997)). Indirect costs are more difficult to estimate. Fractures often lead to limited productivity, which, in some cases, reduces earning potential and the ability to take care of family members. Fractures also impact patients' quality of life and self esteem. For example, hip fracture survivors reported a 52% reduction in quality of life in the 12 months following the fracture (Tosteson et al., *Osteoporos Int.*, 12(12):1042-49 (2001)).

## SUMMARY OF THE INVENTION

**[0005]** The invention is directed to methods of using a sclerostin inhibitor to treat humans with bone fractures. For example, the invention provides a method of treating a bone fracture, wherein the method comprises administering to a subject a therapeutically effective amount of a sclerostin inhibitor during a treatment period lasting at least two weeks. In one aspect, the treatment period begins within two weeks of the fracture. In certain embodiments, the treatment period is about eight weeks, about 4 weeks, or less than 4 weeks, or not longer than 3 weeks, or not longer than 2 weeks (e.g., two weeks), or not longer than 1 week. During the treatment period, the sclerostin inhibitor can be administered once every two weeks, once a week, twice a week, three times a week, or more.

**[0006]** The sclerostin inhibitor may be a sclerostin binding agent (e.g., an anti-sclerostin antibody). The use of sclerostin binding agents disclosed in U.S. Patent Publication No. 20070110747, e.g., in any of the methods disclosed herein or for preparation of medicaments for administration according to any of the methods disclosed herein, is specifically contemplated. One or more doses of the sclerostin inhibitor are administered in an amount and for a time effective to enhance bone fracture healing or improve bone mineral density at the fracture site. One or more doses of sclerostin inhibitor can comprise between about 0.1 to about 30 milligrams of sclerostin inhibitor per kilogram of body weight (mg/kg). For example, the dose of sclerostin inhibitor (e.g., sclerostin binding agent) may range from about 0.5 mg/kg to about 25 mg/kg (e.g., about 0.8 mg/kg to about 20 mg/kg) of body weight. In some embodiments, the dose of sclerostin inhibitor (e.g., sclerostin binding agent) may range from about 0.5 mg/kg to about 10 mg/kg, 1 mg/kg to about 15 mg/kg (e.g., 12 mg/kg), about 1 mg/kg to about 10 mg/kg (e.g., about 2 mg/kg or about 9 mg/kg), or about 3 mg/kg to about 8 mg/kg (e.g., about 4 mg/kg, 5 mg/kg, 6 mg/kg, or 7 mg/kg). In some embodiments, the sclerostin binding agent is administered within two weeks of the fracture, e.g., within 10 days of the fracture, within 7 days of the fracture, within 5 days of the fracture, within 3 days of the fracture, or within 1 day of the fracture.

**[0007]** The invention also includes use of an effective amount of sclerostin binding agent for treating a bone fracture in an amount from about 0.5 mg/kg to about 10 mg/kg, wherein one or more administrations of the sclerostin binding agent is carried out over a treatment period lasting at least two weeks (e.g., two weeks, four weeks, or eight weeks). In some embodiments, the treatment period begins within two weeks of the fracture.

**[0008]** The sclerostin inhibitor may be used in the preparation of a medicament for administration using any of the dosing and timing regimens described herein. Optionally, the sclerostin inhibitor is presented in a container, such as a single dose or multidose vial, containing a dose of sclerostin inhibitor for administration (e.g., about 70 to about 450 mg of sclerostin inhibitor). In one exemplary embodiment, a vial may contain about 70 mg or 75 mg of sclerostin inhibitor, e.g., anti-sclerostin antibody, and would be suitable for administering a single dose of about 1 mg/kg. In other embodiments, a vial may contain about 140 mg or 150 mg; or about 210 mg or 220 mg or 250 mg; or about 280 mg or 290 mg or 300 mg; or about 350 mg or 360 mg; or about 420 mg or 430 mg or 440 mg or 450 mg of sclerostin inhibitor, e.g., anti-sclerostin antibody. The invention includes a container comprising anti-sclerostin antibody or fragment thereof and instructions for administering the antibody or fragment thereof for treating a bone fracture in an amount from about 0.5 mg/kg to about 10 mg/kg twice a week for a treatment period comprising two to four weeks.

## DETAILED DESCRIPTION OF THE INVENTION

**[0009]** The invention is predicated, at least in part, on the discovery that sclerostin inhibitors enhance bone fracture healing as measured in animal models by parameters that indicate increased bone strength to bending and torsional (twisting) forces and by shortened recovery time. In this regard, the invention provides a method of enhancing bone healing or treating a bone fracture. The method comprises administering to a subject (e.g., a mammal, such as a human) one or more doses of a sclerostin inhibitor, such as sclerostin binding agent (e.g., an anti-sclerostin antibody), during a treatment period of, e.g., at least two weeks and/or less than 4 weeks. The materials and methods of the invention are superior to existing therapies whose therapeutic efficacy is limited and require extended recovery time.

**[0010]** Administration of the sclerostin inhibitor enhances or accelerates bone fracture healing, thereby “treating” the bone fracture. “Enhancing” bone healing means mediating a level of bone healing beyond (i.e., greater than) the level of bone healing experienced in subjects (e.g., mammals, such as humans) not administered the sclerostin inhibitor (i.e., control subjects). Bone healing is evidenced by, for example, improved bone density,

improved bone mineral content, bone formation within the fracture gap (i.e., formation of bridging bone), mature bone callus, improved bone strength (optionally accompanied by a medically-acceptable level of bone stiffness), or improved patient use of the affected area. By “improved” is meant an increase or decrease (as desired) in the measured parameter. The increase can be a return, in whole or in part, of the measured parameter to baseline level (e.g., the level prior to the bone fracture), to values provided in normative databases used in the art, or to the contralateral functional level (e.g., return, in whole or in part, to the functional capabilities of, for example, the contralateral limb). In some cases, the increase can be an improvement beyond baseline level. If desired, the measured parameters in patients administered one or more doses of the sclerostin inhibitor can be compared to the same parameters in fracture patients (optionally age and gender matched) not administered the sclerostin inhibitor to further analyze the efficacy of the inventive method.

**[0011]** Bone mineral content, mature boney callus, formation of bridging bone, and bone density at the site of fracture may be measured using single- and/or dual-energy X-ray absorptometry, quantitative computed tomography (QCT), ultrasonography, radiography (e.g., radiographic absorptometry), and magnetic resonance imaging. In some embodiments, the sclerostin inhibitor (e.g., sclerostin binding agent) may be administered at a dose and for a time period effective to increase bone mineral density, bridging bone formation, formation of bony callus, or bone density (or volume) at the fracture site by at least about 5% (about 6%, about 7%, about 8%, or about 9%). In some embodiments, bone mineral density, bridging bone formation, formation of bony callus, or bone density at the fracture site is increased by at least about 10% (e.g., at least about 10%, about 12%, about 15%, or about 18%). In other embodiments, bone mineral density, bridging bone formation, formation of bony callus, or bone density at the fracture site is increased by the sclerostin inhibitor at least about 25% (e.g., at least about 20%, about 22%, about 25%, or about 28%). In yet other embodiments, bone mineral density, bridging bone formation, formation of bony callus, or bone density at the fracture site is increased at least about 30% (e.g., at least about 32%, about 35%, about 38%, or about 40%) or at least about 50% (e.g., at least about 60%, about 70%, about 80%, about 90%, or about 100%). The increase or re-establishment of bone mineral density can be determined at 1 week, 2 weeks, 3 weeks, or 4 weeks following the initial administration of sclerostin inhibitor. Alternatively, the bone density level can be determined after the treatment period ends (e.g., 1 week, 2 weeks, 3 weeks, or 4 weeks after the treatment period). In one aspect, the method reduces the amount of time required to establish a desired level of bone formation, bone volume, bony callus, or bone density (e.g., any percent increase in bone

formation, bone mineral density, bony callus, or bone volume described herein) compared to age and gender-matched patients that do not receive the sclerostin inhibitor, thereby reducing recovery time for a subject. For example, in one embodiment, the sclerostin inhibitor reduces the amount of time required to increase bone density or volume at the site of fracture at least about 10% (e.g., at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45, or at least about 50%).

**[0012]** Functional, quality of life parameters indicative of bone healing include, but are not limited to, recovery of strength and load-bearing capacity, decreased pain and use of pain medication, and improved occupational status. Administration of one or more doses of a sclerostin inhibitor, as described herein, accelerates improvement of functional, quality of life parameters associated with fractures in a statistically significant manner in the patient population tested. In certain aspects, the method reduces recovery time in the patient administered one or more doses of sclerostin inhibitor by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, or at least 65%) compared to recovery time in patients that do not receive the sclerostin inhibitor. “Recovery” can be estimated using any of a number of rehabilitation outcome measurements, such as the FIM instrument motor score for hip fractures (Munin et al., *Arch. Phys. Med. Rehabil.*, 86:367-372 (2005)), the Olerud–Molander Ankle Score (OMAS) and SF-12 questionnaire for ankle fracture (Shah et al., *Injury*, 38(11):1308-1312 (2003)), and Knee Society Scoring for knee replacements (Insall et al., *Clinical Orthopaedics*, 248:13-14 (1989)).

**[0013]** In some embodiments, one or more doses of a sclerostin inhibitor, such as a sclerostin binding agent (e.g., an anti-sclerostin antibody) is administered to a human over the course of a treatment period comprising one, two, three, four, five, six, seven, eight, nine, or ten weeks. A “treatment period” begins upon administration of a first dose of sclerostin inhibitor (e.g., sclerostin binding agent) and ends upon administration of a final dose of sclerostin inhibitor. A dose of sclerostin inhibitor may be administered multiple times per week, if desired. In one embodiment, the treatment period comprises at least two weeks. In some embodiments, the treatment period lasts two, four, or eight weeks. Alternatively or in addition, the treatment period lasts no more than five weeks (e.g., 30 days). Indeed, one or more administrations of a pharmaceutical composition comprising the sclerostin inhibitor may be carried out over a treatment or therapeutic period lasting no more than four weeks (e.g., 26 days or 24 days), less than four weeks, no more than three weeks (e.g., 18 or 15 days), less than three weeks, no more than two weeks (e.g., 12 days or 10 days), or no more than 1 week (e.g., 5 days or 3 days). In one embodiment, the treatment period is not longer

than two weeks, yet yields significant improvement in healing parameters, such as (but not limited to) bone strength (e.g., maximum load-bearing capacity before experiencing pain), bone volume, bridging bone formation, limb function, and/or recovery time, when compared to untreated fractures. For example, after two weeks of treatment, the maximum load sustainable by the patient without pain can be at least about 20% (e.g., 25%, 30%, 35%, or 40%) of pre-fracture strength (or strength of age- and gender-matched subjects). Likewise, after four weeks of treatment, the maximum load sustainable by the patient without pain can be at least about 20% (e.g., 25%, 30%, 35%, or 40%) of pre-fracture strength (or strength of age- and gender-matched subjects, such as subjects untreated for bone fracture). In addition, in one aspect, the treatment period begins soon after a bone fracture is detected, e.g., the treatment period begins within two weeks (e.g., within 10 days, seven days, five days, three days, or one day) of the fracture.

**[0014]** The sclerostin binding agent (e.g., anti-sclerostin antibody) is administered in an amount that promotes, enhances, or accelerates bone fracture healing. The dose of sclerostin binding agent administered to a subject (e.g., a mammal, such as a human) may range from about 0.1 mg/kg to about 30 mg/kg of body weight. For example, the dose of sclerostin binding agent may range from about 0.5 mg/kg to about 25 mg/kg (e.g., about 0.5 mg/kg to about 10 mg/kg, or about 0.8 mg/kg to about 20 mg/kg) of body weight. In some embodiments, the dose of sclerostin binding agent (e.g., anti-sclerostin antibody) may range from about 1 mg/kg to about 15 mg/kg (e.g., 12 mg/kg), about 1 mg/kg to about 10 mg/kg (e.g., about 2 mg/kg or about 9 mg/kg), or about 3 mg/kg to about 8 mg/kg (e.g., about 4 mg/kg, 5 mg/kg, 6 mg/kg, or 7 mg/kg). In one aspect, the dose of sclerostin binding agent is about 0.5 mg/kg to about 2.5 mg/kg (e.g., about 1 mg/kg to about 2 mg/kg, or about 1.5 mg/kg) of body weight. In addition, it may be advantageous to administer multiple doses of a sclerostin binding agent or space out the administration of doses, depending on the therapeutic regimen selected for a particular patient. For example, a dose of sclerostin inhibitor can be administered once every two weeks, once a week, twice a week, three times a week, four times a week, or more, depending on the severity of the fracture, the age and physical health of the patient, and the like.

**[0015]** Bone fractures are classified in a variety of ways. In closed or simple fractures, the skin surrounding the bone is not broken, while open or compound fractures pierce the skin. If the break spans the entire bone, the fracture is “complete.” “Incomplete” or “greenstick” fractures are partial breaks which do not span the entire diameter of the bone. Stress fractures result from the stress of repeated activity that cracks the bone. The inventive method is not

limited to the type of fracture to be treated or the cause of the fracture. For example, the patient with the fracture can also be suffering from a bone related disorder selected from the group consisting of achondroplasia, cleidocranial dysostosis, enchondromatosis, fibrous dysplasia, Gaucher's Disease, hypophosphatemic rickets, Marfan's syndrome, multiple hereditary exostoses, neurofibromatosis, osteogenesis imperfecta, osteopetrosis, osteopoikilosis, sclerotic lesions, pseudoarthrosis, pyogenic osteomyelitis, periodontal disease, anti-epileptic drug induced bone loss, primary and secondary hyperparathyroidism, familial hyperparathyroidism syndromes, weightlessness induced bone loss, osteoporosis in men, postmenopausal bone loss, osteoarthritis, renal osteodystrophy, infiltrative disorders of bone, oral bone loss, osteonecrosis of the jaw, juvenile Paget's disease, melorheostosis, metabolic bone diseases, mastocytosis, sickle cell anemia/disease, organ transplant related bone loss, kidney transplant related bone loss, systemic lupus erythematosus, ankylosing spondylitis, epilepsy, juvenile arthritides, thalassemia, mucopolysaccharidoses, Fabry Disease, Turner Syndrome, Down Syndrome, Klinefelter Syndrome, leprosy, Perthe's Disease, adolescent idiopathic scoliosis, infantile onset multi-system inflammatory disease, Winchester Syndrome, Menkes Disease, Wilson's Disease, ischemic bone disease (such as Legg-Calve-Perthes disease and regional migratory osteoporosis), anemic states, conditions caused by steroids, glucocorticoid-induced bone loss, heparin-induced bone loss, bone marrow disorders, scurvy, malnutrition, calcium deficiency, osteoporosis, osteopenia, alcoholism, chronic liver disease, postmenopausal state, chronic inflammatory conditions, rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, inflammatory colitis, Crohn's disease, oligomenorrhea, amenorrhea, pregnancy, diabetes mellitus, hyperthyroidism, thyroid disorders, parathyroid disorders, Cushing's disease, acromegaly, hypogonadism, immobilization or disuse, reflex sympathetic dystrophy syndrome, regional osteoporosis, osteomalacia, bone loss associated with joint replacement, HIV associated bone loss, bone loss associated with loss of growth hormone, bone loss associated with cystic fibrosis, chemotherapy-associated bone loss, tumor-induced bone loss, cancer-related bone loss, hormone ablative bone loss, multiple myeloma, drug-induced bone loss, anorexia nervosa, disease-associated facial bone loss, disease-associated cranial bone loss, disease-associated bone loss of the jaw, disease-associated bone loss of the skull, bone loss associated with aging, facial bone loss associated with aging, cranial bone loss associated with aging, jaw bone loss associated with aging, skull bone loss associated with aging, and bone loss associated with space travel

**[0016]** The sclerostin binding agent is preferably administered to a patient in a physiologically-acceptable (e.g., pharmaceutical) composition, which can include carriers, excipients, or diluents. It will be appreciated that the sclerostin binding agents described herein may be used in the preparation of a medicament for administration using any of the dosage and timing regimens disclosed herein. Pharmaceutical compositions and methods of treatment are disclosed in U.S. Patent Publication No. 20050106683, which is incorporated by reference herein. “Physiologically-acceptable” refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. In addition, the composition administered to a subject may contain more than one sclerostin inhibitor (e.g., two anti-sclerostin antibodies, or a sclerostin binding agent and a synthetic chemical sclerostin inhibitor) or a sclerostin inhibitor in combination with one or more therapeutics having different mechanisms of action.

**[0017]** The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., subcutaneous, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art and discussed in U.S. Patent Publication No. 20070110747. For example, in certain circumstances, it will be desirable to deliver a pharmaceutical composition comprising a sclerostin binding agent subcutaneously, parenterally, intravenously, intramuscularly, or even intraperitoneally. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U.S. Patent Nos. 5,543,158; 5,641,515; and 5,399,363. Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U.S. Patent No. 5,466,468). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists.

**[0018]** In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion (see, for example, Remington's Pharmaceutical Sciences, 15th ed., Mack Pub. Co., Easton, PA, pp. 1035-1038 and 1570-1580). Some variation in dosage and frequency of administration may occur depending on the condition of the subject being treated; age, height, weight, and overall

health of the patient; and the existence of any side effects. In addition, a pharmaceutical composition comprising a sclerostin binding agent may be placed within containers (e.g. vials), along with packaging material that provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) that may be necessary to reconstitute the pharmaceutical composition.

**[0019]** The inventive method comprises administering an amount of a “sclerostin inhibitor.” As used herein, the term “sclerostin inhibitor” means any molecule that inhibits the biological activity of sclerostin on bone, as measured by changes to bone mineralization, bone density, effect on osteoblasts and/or osteoclasts, markers of bone formation, markers of bone resorption, markers of osteoblast activity, and/or markers of osteoclast activity. Such inhibitors may act by binding to sclerostin or its receptor or binding partner. Inhibitors in this category include “sclerostin binding agents,” such as, e.g., antibodies or peptide-based molecules. “Sclerostin inhibitors” also refers to small organic chemical compounds, optionally of less than about 1000 Daltons in molecular weight that bind sclerostin and inhibit its activity. Inhibitors may alternatively act by inhibiting expression of sclerostin. Inhibitors in this category include polynucleotides or oligonucleotides that bind to sclerostin DNA or mRNA and inhibit sclerostin expression, including an antisense oligonucleotide, inhibitory RNA, DNA enzyme, ribozyme, an aptamer or pharmaceutically acceptable salts thereof that inhibit the expression of sclerostin.

**[0020]** A “sclerostin binding agent” specifically binds to sclerostin or portions thereof to block or impair binding of human sclerostin to one or more ligands. Sclerostin, the product of the SOST gene, is absent in sclerosteosis, a skeletal disease characterized by bone overgrowth and strong dense bones (Brunkow et al., *Am. J. Hum. Genet.*, 68:577-589 (2001); Balemans et al., *Hum. Mol. Genet.*, 10:537-543 (2001)). The amino acid sequence of human sclerostin is reported by Brunkow et al. and is disclosed in U.S. Patent Publication No. 20070110747 as SEQ ID NO: 1 (which patent publication is incorporated in its entirety for its description of sclerostin binding agents and Sequence Listing). Recombinant human sclerostin/SOST is commercially available from R&D Systems (Minneapolis, Minn., USA; 2006 Catalog #1406-ST-025). Additionally, recombinant mouse sclerostin/SOST is commercially available from R&D Systems (Minneapolis, Minn., USA; 2006 Catalog #1589-ST-025). Research grade sclerostin-binding monoclonal antibodies are commercially available from R&D Systems (Minneapolis, Minn., USA; mouse monoclonal: 2006 Catalog #

MAB1406; rat monoclonal: 2006 Catalog # MAB1589). U.S. Patent Nos. 6,395,511 and 6,803,453, and U.S. Patent Publication Nos. 20040009535 and 20050106683 refer to anti-sclerostin antibodies generally. Examples of sclerostin binding agents suitable for use in the context of the invention also are described in U.S. Patent Publication Nos. 20070110747 and 20070072797, which are hereby incorporated by reference. Additional information regarding materials and methods for generating sclerostin binding agents can be found in U.S. Patent Publication No. 20040158045.

**[0021]** The sclerostin binding agent of the invention preferably is an antibody. The term "antibody" refers to an intact antibody or a binding fragment thereof. An antibody may comprise a complete antibody molecule (including polyclonal, monoclonal, chimeric, humanized, or human versions having full length heavy and/or light chains), or comprise an antigen binding fragment thereof. Antibody fragments include  $F(ab')_2$ , Fab, Fab', Fv, Fc, and Fd fragments, and can be incorporated into single domain antibodies, single-chain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, *Nature Biotechnology*, 23(9):1126-1136 (2005)). Antibody polypeptides, including fibronectin polypeptide monobodies, also are disclosed in U.S. Patent No. 6,703,199. Other antibody polypeptides are disclosed in U.S. Patent Publication No. 20050238646. Anti-sclerostin antibodies may bind to sclerostin of SEQ ID NO: 1, or a naturally occurring variant thereof, with an affinity of less than or equal to  $1 \times 10^{-7} M$ , less than or equal to  $1 \times 10^{-8} M$ , less than or equal to  $1 \times 10^{-9} M$ , less than or equal to  $1 \times 10^{-10} M$ , less than or equal to  $1 \times 10^{-11} M$ , or less than or equal to  $1 \times 10^{-12} M$ . Affinity may be determined by an affinity ELISA assay. In certain embodiments, affinity may be determined by a BIACore assay. In certain embodiments, affinity may be determined by a kinetic method. In certain embodiments, affinity may be determined by an equilibrium/solution method.

**[0022]** An antibody fragment may be any synthetic or genetically engineered protein. For example, antibody fragments include isolated fragments consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, and recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (scFv proteins).

**[0023]** Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs (also termed "minimal recognition units" or "hypervariable region") can be obtained by constructing polynucleotides that encode the CDR of interest. Such polynucleotides are prepared, for example, by using

the polymerase chain reaction to synthesize the variable region using mRNA of antibody-producing cells as a template (see, for example, Larrick et al., *Methods: A Companion to Methods in Enzymology*, 2:106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies Production, Engineering and Clinical Application*, Ritter et al. (eds.), page 166, Cambridge University Press (1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch et al., (eds.), page 137, Wiley-Liss, Inc. (1995)).

**[0024]** In one embodiment of the invention, the sclerostin binding agent cross-blocks the binding of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24 (all of which are described in U.S. Patent Publication No. 20070110747) to sclerostin. Alternatively or in addition, the sclerostin binding agent is cross-blocked from binding to sclerostin by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24 (all of which are described in U.S. Patent Publication No. 20070110747). The terms "cross-block," "cross-blocked," and "cross-blocking" are used interchangeably herein to mean the ability of an antibody or other binding agent to interfere with the binding of other antibodies or binding agents to sclerostin. The extent to which an antibody or other binding agent is able to interfere with the binding of another to sclerostin, and therefore whether it can be said to cross-block, can be determined using competition binding assays. In some aspects of the invention, a cross-blocking antibody or fragment thereof reduces sclerostin binding of a reference antibody between about 40% and about 100%, such as about 60% and about 100%, specifically between 70% and 100%, and more specifically between 80% and 100%. A particularly suitable quantitative assay for detecting cross-blocking uses a Biacore machine which measures the extent of interactions using surface plasmon resonance technology. Another suitable quantitative cross-blocking assay uses an ELISA-based approach to measure competition between antibodies or other binding agents in terms of their binding to sclerostin.

**[0025]** Suitable sclerostin binding agents include antibodies and portions thereof described in U.S. Patent Publication No. 20070110747, such as one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 as specifically disclosed therein. At least one of the regions of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 may have at least one amino acid substitution, provided that the binding agent retains the binding

specificity of the non-substituted CDR. The non-CDR portion of the binding agent may be a non-protein molecule, wherein the binding agent cross-blocks the binding of an antibody disclosed herein to sclerostin and/or neutralizes sclerostin. The non-CDR portion of the binding agent may be a non-protein molecule in which the binding agent exhibits a similar binding pattern to human sclerostin peptides in a human sclerostin peptide epitope competition binding assay as that exhibited by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24 (all of which are described in U.S. Patent Publication No. 20070110747), and/or neutralizes sclerostin. The non-CDR portion of the binding agent may be composed of amino acids, wherein the binding agent is a recombinant binding protein or a synthetic peptide, and the recombinant binding protein cross-blocks the binding of an antibody to sclerostin and/or neutralizes sclerostin. The non-CDR portion of the binding agent may be composed of amino acids, wherein the binding agent is a recombinant binding protein, and the recombinant binding protein exhibits a similar binding pattern to human sclerostin peptides in the human sclerostin peptide epitope competition binding assay (described in U.S. Patent Publication No. 20070110747) as that exhibited by at least one of the antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24 (described in U.S. Patent Publication No. 20070110747), and/or neutralizes sclerostin. Preferably, the sclerostin binding agent is Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, or Ab-24 of U.S. Patent Publication No. 20070110747.

**[0026]** In addition, the sclerostin binding agent can comprise at least one CDR sequence having at least 75% identity (e.g., 100% identity) to a CDR selected from SEQ ID NOs: 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 78, 79, 80, 81, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 351, 352, 353, 358, 359, and 360 disclosed in U.S. Patent Publication No. 20070110747. Preferably, the sclerostin binding agent comprises at least one CDR sequence having at least 75% identity to a CDR selected from SEQ ID

NOs: 245, 246, 247, 78, 79, 80, 269, 270, 271, 239, 240, and 241, all of which is described in U.S. Patent Publication No. 20070110747. As described in U.S. Patent Publication No. 20070110747, the sclerostin binding agent can comprise: a) CDR sequences of SEQ ID NOs:54, 55, and 56 and CDR sequences of SEQ ID NOs:51, 52, and 53; b) CDR sequences of SEQ ID NOs:60, 61, and 62 and CDR sequences of SEQ ID NOs:57, 58, and 59; c) CDR sequences of SEQ ID NOs:48, 49, and 50 and CDR sequences of SEQ ID NOs:45, 46, and 47; d) CDR sequences of SEQ ID NOs:42, 43, and 44 and CDR sequences of SEQ ID NOs:39, 40, and 41; e) CDR sequences of SEQ ID NOs:275, 276, and 277 and CDR sequences of SEQ ID NOs:287, 288, and 289; f) CDR sequences of SEQ ID NOs:278, 279, and 280 and CDR sequences of SEQ ID NOs:290, 291, and 292; g) CDR sequences of SEQ ID NOs:78, 79, and 80 and CDR sequences of SEQ ID NOs: 245, 246, and 247; h) CDR sequences of SEQ ID NOs:81, 99, and 100 and CDR sequences of SEQ ID NOs:248, 249, and 250; i) CDR sequences of SEQ ID NOs:101, 102, and 103 and CDR sequences of SEQ ID NOs:251, 252, and 253; j) CDR sequences of SEQ ID NOs:104, 105, and 106 and CDR sequences of SEQ ID NOs:254, 255, and 256; k) CDR sequences of SEQ ID NOs:107, 108, and 109 and CDR sequences of SEQ ID NOs:257, 258, and 259; l) CDR sequences of SEQ ID NOs:110, 111, and 112 and CDR sequences of SEQ ID NOs:260, 261, and 262; m) CDR sequences of SEQ ID NOs:281, 282, and 283 and CDR sequences of SEQ ID NOs:293, 294, and 295; n) CDR sequences of SEQ ID NOs:113, 114, and 115 and CDR sequences of SEQ ID NOs:263, 264, and 265; o) CDR sequences of SEQ ID NOs:284, 285, and 286 and CDR sequences of SEQ ID NOs:296, 297, and 298; p) CDR sequences of SEQ ID NOs:116, 237, and 238 and CDR sequences of SEQ ID NOs:266, 267, and 268; q) CDR sequences of SEQ ID NOs:239, 240, and 241 and CDR sequences of SEQ ID NOs:269, 270, and 271; r) CDR sequences of SEQ ID NOs:242, 243, and 244 and CDR sequences of SEQ ID NOs:272, 273, and 274; or s) CDR sequences of SEQ ID NOs:351, 352, and 353 and CDR sequences of SEQ ID NOs:358, 359, and 360.

**[0027]** The sclerostin binding agent also can comprise at least one CDR sequence having at least 75% identity (e.g., 100% identity) to a CDR selected from CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 wherein CDR-H1 has the sequence given in SEQ ID NO: 245 or SEQ ID NO: 269, CDR-H2 has the sequence given in SEQ ID NO: 246 or SEQ ID NO: 270, CDR-H3 has the sequence given in SEQ ID NO: 247 or SEQ ID NO: 271, CDR-L1 has the sequence given in SEQ ID NO: 78 or SEQ ID NO: 239, CDR-L2 has the sequence given in SEQ ID NO: 79 or SEQ ID NO: 240 and CDR-L3 has the sequence given

in SEQ ID NO: 80 or SEQ ID NO: 241, all of which is described in U.S. Patent Publication No. 20070110747.

**[0028]** Alternatively, the sclerostin binding agent can have a heavy chain comprising CDR's H1, H2, and H3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 137 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 245, 246, and 247, respectively, and a light chain comprising CDR's L1, L2 and L3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 133 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 78, 79, and 80, respectively (as described in U.S. Patent Publication No. 20070110747).

**[0029]** The sclerostin binding agent may have a heavy chain comprising CDR's H1, H2, and H3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 145 or 392 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 245, 246, and 247, respectively, and a light chain comprising CDR's L1, L2, and L3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 141 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 78, 79, and 80, respectively (as described in U.S. Patent Publication No. 20070110747).

**[0030]** The sclerostin binding agent may have a heavy chain comprising CDR's H1, H2, and H3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 335 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 269, 270, and 271, respectively, and a light chain comprising CDR's L1, L2, and L3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 334 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 239, 240, and 241, respectively (as described in U.S. Patent Publication No. 20070110747).

**[0031]** Alternatively, the sclerostin binding agent has a heavy chain comprising CDR's H1, H2, and H3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 331 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 269, 270, and 271, respectively, and a light chain comprising CDR's L1, L2, and L3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 330 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 239, 240, and 241, respectively (as described in U.S. Patent Publication No. 20070110747).

**[0032]** The sclerostin binding agent may have a heavy chain comprising CDR's H1, H2, and H3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 345 or 396 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 269, 270, and 271, respectively, and a light chain comprising CDR's L1, L2, and L3 and comprising a polypeptide having the sequence provided in SEQ ID NO: 341 or a variant thereof in which said CDR's are at least 75% identical (e.g., 100% identical) to SEQ ID NO: 239, 240, and 241, respectively (as described in U.S. Patent Publication No. 20070110747).

**[0033]** Alternatively, the sclerostin binding agent has a heavy chain comprising a polypeptide having the sequence provided in SEQ ID NO: 137, and a light chain comprising a polypeptide having the sequence provided in SEQ ID NO: 133; or a heavy chain comprising a polypeptide having the sequence provided in SEQ ID NO: 145 or 392, and a light chain comprising a polypeptide having the sequence provided in SEQ ID NO: 141; or a heavy chain comprising a polypeptide having the sequence provided in SEQ ID NO: 335, and a light chain comprising a polypeptide having the sequence provided in SEQ ID NO: 334; or a heavy chain comprising a polypeptide having the sequence provided in SEQ ID NO: 331, and a light chain comprising a polypeptide having the sequence provided in SEQ ID NO: 330; or a heavy chain comprising a polypeptide having the sequence provided in SEQ ID NO: 345 or 396, and a light chain comprising a polypeptide having the sequence provided in SEQ ID NO: 341 (as described in U.S. Patent Publication No. 20070110747).

**[0034]** Sclerostin inhibitors (e.g., sclerostin binding agents) for use in the inventive method preferably modulate sclerostin function in the cell-based assay described in U.S. Patent Publication No. 20070110747 and/or the *in vivo* assay described in U.S. Patent Publication No. 20070110747 and/or bind to one or more of the epitopes described in U.S. Patent Publication No. 20070110747 and/or cross-block the binding of one of the antibodies described in U.S. Patent Publication No. 20070110747 and/or are cross-blocked from binding sclerostin by one of the antibodies described in U.S. Patent Publication No. 20070110747.

**[0035]** Alternatively, the inventive method can comprise administering a sclerostin inhibitor other than a sclerostin binding agent. Such agents can act directly or indirectly on SOST or sclerostin. Sclerostin inhibitors contemplated for use in the inventive method include those described in U.S. Patent Publication No. 20030229041 (the entire disclosure of which is hereby incorporated by reference, with particular emphasis upon the description of sclerostin inhibitors). For example, agents useful for modulating SOST expression and sclerostin activity include, but are not limited to, steroids (such as those corresponding to

Formula 1 of U.S. Patent Publication No. 20030229041), alkaloids, terpenoids, peptoids, and synthetic chemicals. In some embodiments, the SOST antagonist or agonist can bind to a glucocorticoid receptor. For example, dexamethasone tends to abolish the stimulatory effect of BMP-4 and BMP-6 on SOST expression. Other chemical entities including glucocorticoid analogs, bile salts (such as those corresponding to Formula 3 of U.S. Patent Publication No. 20030229041), and prostaglandins (such as those corresponding to Formula 2 of U.S. Patent Publication No. 20030229041) also modulate the effects of bone morphogenetic proteins on SOST expression, and are contemplated for use in the inventive method.

**[0036]** The sclerostin inhibitor may also be other small molecule therapeutics that act directly or indirectly on SOST or sclerostin to accelerate or enhance bone fracture repair *in vivo*. The term "small molecule" includes a compound or molecular complex, either synthetic, naturally derived, or partially synthetic, and which preferably has a molecular weight of less than 5,000 Daltons (e.g., between about 100 and 1,500 Daltons). Agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection (see, e.g., Lam, *Anticancer Drug Des.*, 12:145 (1997) and U.S. Patent Nos. 5,738,996; 5,807,683; and 7,261,892). Methods of developing and screening sclerostin inhibitors are further described in U.S. Patent Publication No. 20030229041, the discussion of which is hereby incorporated by reference.

**[0037]** Sclerostin expression inhibitors that may be used according to the methods of the invention include inhibitor oligonucleotides or polynucleotides, including pharmaceutically acceptable salts thereof, e.g., sodium salts. Nonlimiting examples include: antisense oligonucleotides (Eckstein, *Antisense Nucleic Acid Drug Dev.*, 10:117-121 (2000); Crooke, *Methods Enzymol.*, 313:3-45 (2000); Guvakova et al., *J. Biol. Chem.*, 270:2620-2627 (1995); Manoharan, *Biochim. Biophys. Acta*, 1489:117-130 (1999); Baker et al., *J. Biol. Chem.*, 272:11994-12000 (1997); Kurreck, *Eur. J. Biochem.*, 270:1628-1644 (2003); Sierakowska et al., *Proc. Natl. Acad. Sci. USA*, 93:12840-12844 (1996); Marwick, *J. Am. Med. Assoc.*, 280:871 (1998); Tomita and Morishita, *Curr. Pharm. Des.*, 10:797-803 (2004); Gleave and Monia, *Nat. Rev. Cancer*, 5:468-479 (2005) and Patil, *AAPS J.*, 7:E61-E77 (2005)), triplex oligonucleotides (Francois et al., *Nucleic Acids Res.*, 16:11431-11440 (1988) and Moser and Dervan, *Science*, 238:645-650 (1987)), ribozymes/deoxyribozymes (DNAzymes) (Kruger et al., *Tetrahymena. Cell*, 31:147-157 (1982); Uhlenbeck, *Nature*, 328:596-600 (1987);

Sigurdsson and Eckstein, *Trends Biotechnol.*, 13:286-289 (1995); Kumar et al., *Gene Ther.*, 12:1486-1493 (2005); Breaker and Joyce, *Chem. Biol.*, 1:223-229 (1994); Khachigian, *Curr. Pharm. Biotechnol.*, 5:337-339 (2004); Khachigian, *Biochem. Pharmacol.*, 68:1023-1025 (2004) and Trulzsch and Wood, *J. Neurochem.*, 88:257-265 (2004)), small-interfering RNAs/RNAi (Fire et al., *Nature*, 391:806-811 (1998); Montgomery et al., *Proc. Natl. Acad. Sci. U.S.A.*, 95:15502-15507 (1998); Cullen, *Nat. Immunol.*, 3:597-599 (2002); Hannon, *Nature*, 418:244-251 (2002); Bernstein et al., *Nature*, 409:363-366 (2001); Nykanen et al., *Cell*, 107:309-321 (2001); Gilmore et al., *J. Drug Target.*, 12:315-340 (2004); Reynolds et al., *Nat. Biotechnol.*, 22:326-330 (2004); Soutschek et al., *Nature*, 432:173-178 (2004); Ralph et al., *Nat. Med.*, 11:429-433 (2005); Xia et al., *Nat. Med.*, 10(8):816-820 (2004) and Miller et al., *Nucleic Acids Res.*, 32:661-668 (2004)), aptamers (Ellington and Szostak, *Nature*, 346:818-822 (1990); Doudna et al., *Proc. Natl. Acad. Sci. U.S.A.*, 92:2355-2359 (1995); Tuerk and Gold, *Science*, 249:505-510 (1990); White et al., *Mol. Ther.*, 4:567-573 (2001); Rusconi et al., *Nature*, 419:90-94 (2002); Nimjee et al., *Mol. Ther.*, 14:408-415 (2006); Gragoudas et al., *N. Engl. J. Med.*, 351:3805-2816 (2004); Vinores, *Curr. Opin. Mol. Ther.*, 5(6):673-679 (2003) and Kourlas and Schiller et al., *Clin. Ther.*, 28:36-44 (2006)) or decoy oligonucleotides (Morishita et al., *Proc. Natl. Acad. Sci. U.S.A.*, 92:5855-5859 (1995); Alexander et al., *J. Am. Med. Assoc.*, 294:2446-2454 (2005); Mann and Dzau, *J. Clin. Invest.*, 106:1071-1075 (2000) and Nimjee et al., *Annu. Rev. Med.*, 56:555-583 (2005)). The foregoing documents are hereby incorporated by reference in their entirety herein, with particular emphasis on those sections of the documents relating to methods of designing, making and using inhibitory oligonucleotides. Commercial providers such as Ambion Inc. (Austin, TX), Darmacon Inc. (Lafayette, CO), InvivoGen (San Diego, CA), and Molecular Research Laboratories, LLC (Herndon, VA) generate custom siRNA molecules. In addition, commercial kits are available to produce custom siRNA molecules, such as SILENCER™ siRNA Construction Kit (Ambion Inc., Austin, TX) or psiRNA System (InvivoGen, San Diego, CA).

**[0038]** Inhibitory oligonucleotides which are stable, have a high resistance to nucleases, possess suitable pharmacokinetics to allow them to traffic to target tissue site at non-toxic doses, and have the ability to cross through plasma membranes are contemplated for use as a therapeutic. Inhibitory oligonucleotides may be complementary to the coding portion of a target gene, 3' or 5' untranslated regions, or intronic sequences in a gene, or alternatively coding or intron sequences in the target mRNA. Intron sequences are generally less conserved and thus may provide greater specificity. In one embodiment, the inhibitory

oligonucleotide inhibits expression of a gene product of one species but not its homologue in another species; in other embodiments, the inhibitory oligonucleotide inhibits expression of a gene in two species, e.g. human and primate, or human and murine.

**[0039]** The constitutive expression of antisense oligonucleotides in cells has been shown to inhibit gene expression, possibly via the blockage of translation or prevention of splicing. In certain embodiments, the inhibitory oligonucleotide is capable of hybridizing to at least 8, 9, 10, 11, or 12 consecutive bases of the sclerostin gene or mRNA (or the reverse strand thereof) under moderate or high stringency conditions. Suitable inhibitory oligonucleotides may be single stranded and contain a segment, e.g., at least 12, 15 or 18 bases in length, that is sufficiently complementary to, and specific for, an mRNA or DNA molecule such that it hybridizes to the mRNA or DNA molecule and inhibits transcription, splicing, or translation. Generally complementarity over a length of less than 30 bases is more than sufficient.

**[0040]** Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short nucleic acids (e.g., 10 to 50 nucleotides) and at least about 60°C for longer nucleic acids (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30% to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50°C to 55°C. Exemplary moderate stringency conditions include hybridization in 40% to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55°C to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60°C to 65°C. Duration of hybridization is generally less than about 24 hours, usually about 4 hours to about 12 hours.

**[0041]** In some cases, depending on the length of the complementary region, one, two or more mismatches may be tolerated without affecting inhibitory function. In certain embodiments, the inhibitory oligonucleotide is an antisense oligonucleotide, an inhibitory RNA (including siRNA or RNAi, or shRNA), a DNA enzyme, a ribozyme (optionally a hammerhead ribozyme), an aptamer, or pharmaceutically acceptable salts thereof. In one embodiment, the oligonucleotide is complementary to at least 10 bases of the nucleotide sequence encoding SEQ ID NO: 1 of U.S. Patent Publication No. 20040158045. In one embodiment, the oligonucleotide targets the nucleotides located in the vicinity of the 3' untranslated region of the sclerostin mRNA.

**[0042]** The specific sequence utilized in design of the oligonucleotides may be any contiguous sequence of nucleotides contained within the expressed gene message of the target. Factors that govern a target site for the inhibitory oligonucleotide sequence include the length of the oligonucleotide, binding affinity, and accessibility of the target sequence. Sequences may be screened *in vitro* for potency of their inhibitory activity by measuring inhibition of target protein translation and target related phenotype, e.g., inhibition of cell proliferation in cells in culture. In general it is known that most regions of the RNA (5' and 3' untranslated regions, AUG initiation, coding, splice junctions and introns) can be targeted using antisense oligonucleotides. Programs and algorithms, known in the art, may be used to select appropriate target sequences. In addition, optimal sequences may be selected utilizing programs designed to predict the secondary structure of a specified single stranded nucleic acid sequence and allowing selection of those sequences likely to occur in exposed single stranded regions of a folded mRNA. Methods and compositions for designing appropriate oligonucleotides may be found, for example, in U.S. Patent No. 6,251,588, the contents of which are incorporated herein by reference in its entirety.

**[0043]** Phosphorothioate antisense oligonucleotides may be used. Modifications of the phosphodiester linkage as well as of the heterocycle or the sugar may provide an increase in efficiency. Phosphorothioate is used to modify the phosphodiester linkage. An N3'-P5' phosphoramidate linkage has been described as stabilizing oligonucleotides to nucleases and increasing the binding to RNA. Peptide nucleic acid (PNA) linkage is a complete replacement of the ribose and phosphodiester backbone and is stable to nucleases, increases the binding affinity to RNA, and does not allow cleavage by RNase H. Its basic structure is also amenable to modifications that may allow its optimization as an antisense component. With respect to modifications of the heterocycle, certain heterocycle modifications have proven to augment antisense effects without interfering with RNase H activity. An example of such modification is C-5 thiazole modification. Finally, modification of the sugar may also be considered. 2'-O-propyl and 2'-methoxyethoxy ribose modifications stabilize oligonucleotides to nucleases in cell culture and *in vivo*.

**[0044]** Most mRNAs have been shown to contain a number of secondary and tertiary structures. Secondary structural elements in RNA are formed largely by Watson-Crick type interactions between different regions of the same RNA molecule. Important secondary structural elements include intramolecular double stranded regions, hairpin loops, bulges in duplex RNA and internal loops. Tertiary structural elements are formed when secondary structural elements come in contact with each other or with single stranded regions to

produce a more complex three dimensional structure. A number of researchers have measured the binding energies of a large number of RNA duplex structures and have derived a set of rules which can be used to predict the secondary structure of RNA (see e.g. Jaeger et al., *Proc. Natl. Acad. Sci. USA*, 86(20):7706-10 (1989); and Turner et al., *Annu. Rev. Biophys. Biophys. Chem.*, 17:167-192 (1988)). The rules are useful in identification of RNA structural elements and, in particular, for identifying single stranded RNA regions which may represent segments of the mRNA to target for siRNA, ribozyme, or antisense technologies.

**[0045]** Short interfering (si) RNA technology (also known as RNAi) generally involves degradation of an mRNA of a particular sequence induced by double-stranded RNA (dsRNA) that is homologous to that sequence, thereby “interfering” with expression of the corresponding gene. Any selected gene may be repressed by introducing a dsRNA which corresponds to all or a substantial part of the mRNA for that gene. It appears that when a long dsRNA is expressed, it is initially processed by a ribonuclease III into shorter dsRNA oligonucleotides of as few as 21 to 22 base pairs in length. Accordingly, siRNA may be affected by introduction or expression of relatively short homologous dsRNAs. Exemplary siRNAs have sense and antisense strands of about 21 nucleotides that form approximately 19 nucleotide of double stranded RNA with overhangs of two nucleotides at each 3' end. Indeed the use of relatively short homologous dsRNAs may have certain advantages.

**[0046]** Mammalian cells have at least two pathways that are affected by double-stranded RNA (dsRNA). In the sequence-specific siRNA pathway, the initiating dsRNA is first broken into short interfering RNAs, as described above. Short interfering RNAs are thought to provide the sequence information that allows a specific messenger RNA to be targeted for degradation. In contrast, the nonspecific pathway is triggered by dsRNA of any sequence, as long as it is at least about 30 base pairs in length.

**[0047]** The nonspecific effects occur because dsRNA activates two enzymes: PKR, which in its active form phosphorylates the translation initiation factor eIF2 to shut down all protein synthesis, and 2', 5' oligoadenylate synthetase (2', 5'-AS), which synthesizes a molecule that activates RNase L, a nonspecific enzyme that targets all mRNAs. The nonspecific pathway may represent a host response to stress or viral infection, and, in general, the effects of the nonspecific pathway are preferably minimized. Significantly, longer dsRNAs appear to be required to induce the nonspecific pathway and, accordingly, dsRNAs shorter than about 30 bases pairs are contemplated to effect gene repression by RNAi (see Hunter et al., *J. Biol. Chem.*, 250:409-17 (1975); Manche et al., *Mol. Cell. Biol.* 12:5239-48 (1992); Minks et al., *J. Biol. Chem.*, 254:10180-3 (1979); and Elbashir et al., *Nature*, 411:494-8 (2001)).

**[0048]** siRNA has proven to be an effective means of decreasing gene expression in a variety of cell types. siRNA typically decreases expression of a gene to lower levels than that achieved using antisense techniques, and frequently eliminates expression entirely (see Bass, *Nature*, 411: 428-9 (2001)). In mammalian cells, siRNAs are effective at concentrations that are several orders of magnitude below the concentrations typically used in antisense experiments (Elbashir et al., *Nature*, 411:494-8 (2001)).

**[0049]** The double stranded oligonucleotides used to effect RNAi are preferably less than 30 base pairs in length, for example, about 25, 24, 23, 22, 21, 20, 19, 18, or 17 base pairs or less in length, and contain a segment sufficiently complementary to the target mRNA to allow hybridization to the target mRNA. Optionally the dsRNA oligonucleotides may include 3' overhang ends. Exemplary 2-nucleotide 3' overhangs may be composed of ribonucleotide residues of any type and may even be composed of 2'-deoxythymidine residues, which lowers the cost of RNA synthesis and may enhance nuclease resistance of siRNAs in the cell culture medium and within transfected cells (see Elbashi et al., *supra*). Exemplary dsRNAs may be synthesized chemically or produced *in vitro* or *in vivo* using appropriate expression vectors (see, e.g., Elbashir et al., *Genes Dev.*, 15:188-200 (2001)). Longer RNAs may be transcribed from promoters, such as T7 RNA polymerase promoters, known in the art.

**[0050]** Longer dsRNAs of 50, 75, 100, or even 500 base pairs or more also may be utilized in certain embodiments of the invention. Exemplary concentrations of dsRNAs for effecting RNAi are about 0.05 nM, 0.1 nM, 0.5 nM, 1.0 nM, 1.5 nM, 25 nM, or 100 nM, although other concentrations may be utilized depending upon the nature of the cells treated, the gene target and other factors readily discernable to the skilled artisan.

**[0051]** Further compositions, methods and applications of siRNA technology are provided in U.S. Patent Nos. 6,278,039; 5,723,750; and 5,244,805, which are incorporated herein by reference in its entirety.

**[0052]** Compared to siRNA, shRNA offers advantages in silencing longevity and delivery options. See, e.g., Hannon et al., *Nature*, 431:371-378 (2004) for review. Vectors that produce shRNAs, which are processed intracellularly into short duplex RNAs having siRNA-like properties have been reported (Brummelkamp et al., *Science*, 296:550-553 (2000); Paddison et al., *Genes Dev.*, 16: 948-958 (2002)). Such vectors provide a renewable source of a gene-silencing reagent that can mediate persistent gene silencing after stable integration of the vector into the host-cell genome. Furthermore, the core silencing 'hairpin' cassette can

be readily inserted into retroviral, lentiviral, or adenoviral vectors, facilitating delivery of shRNAs into a broad range of cell types (Brummelkamp et al., *Cancer Cell*, 2:243–247 (2002); Dirac et al., *J. Biol. Chem.*, 278:11731–11734 (2003); Michiels et al., *Nat. Biotechnol.*, 20:1154–1157 (2002); Stegmeier et al., *Proc. Natl. Acad. Sci. USA*, 102:13212–13217 (2005); Khvorova et al., *Cell*, 115:209–216 (2003)) in any of the innumerable ways that have been devised for delivery of DNA constructs that allow ectopic mRNA expression.

**[0053]** A hairpin can be organized in either a left-handed hairpin (i.e., 5'-antisense-loop-sense-3') or a right-handed hairpin (i.e., 5'-sense-loop-antisense-3'). The siRNA may also contain overhangs at either the 5' or 3' end of either the sense strand or the antisense strand, depending upon the organization of the hairpin. Preferably, if there are any overhangs, they are on the 3' end of the hairpin and comprise between 1 to 6 bases. The overhangs can be unmodified, or can contain one or more specificity or stabilizing modifications, such as a halogen or O-alkyl modification of the 2' position, or internucleotide modifications such as phosphorothioate, phosphorodithioate, or methylphosphonate modifications. The overhangs can be ribonucleic acid, deoxyribonucleic acid, or a combination of ribonucleic acid and deoxyribonucleic acid.

**[0054]** Additionally, a hairpin can further comprise a phosphate group on the 5'-most nucleotide. The phosphorylation of the 5'-most nucleotide refers to the presence of one or more phosphate groups attached to the 5' carbon of the sugar moiety of the 5'-terminal nucleotide. Preferably, there is only one phosphate group on the 5' end of the region that will form the antisense strand following Dicer processing. In one exemplary embodiment, a right-handed hairpin can include a 5' end (i.e., the free 5' end of the sense region) that does not have a 5' phosphate group, or can have the 5' carbon of the free 5'-most nucleotide of the sense region being modified in such a way that prevents phosphorylation. This can be achieved by a variety of methods including, but not limited to, addition of a phosphorylation blocking group (e.g., a 5'-O-alkyl group), or elimination of the 5'-OH functional group (e.g., the 5'-most nucleotide is a 5'-deoxy nucleotide). In cases where the hairpin is a left-handed hairpin, preferably the 5' carbon position of the 5'-most nucleotide is phosphorylated.

**[0055]** Hairpins that have stem lengths longer than 26 base pairs can be processed by Dicer such that some portions are not part of the resulting siRNA that facilitates mRNA degradation. Accordingly the first region, which may comprise sense nucleotides, and the second region, which may comprise antisense nucleotides, may also contain a stretch of nucleotides that are complementary (or at least substantially complementary to each other), but are or are not the same as or complementary to the target mRNA. While the stem of the

shRNA can be composed of complementary or partially complementary antisense and sense strands exclusive of overhangs, the shRNA can also include the following: (1) the portion of the molecule that is distal to the eventual Dicer cut site contains a region that is substantially complementary/homologous to the target mRNA; and (2) the region of the stem that is proximal to the Dicer cut site (i.e., the region adjacent to the loop) is unrelated or only partially related (e.g., complementary/homologous) to the target mRNA. The nucleotide content of this second region can be chosen based on a number of parameters including but not limited to thermodynamic traits or profiles.

**[0056]** Modified shRNAs can retain the modifications in the post-Dicer processed duplex. In exemplary embodiments, in cases in which the hairpin is a right handed hairpin (e.g., 5'-S-loop-AS-3') containing 2-6 nucleotide overhangs on the 3' end of the molecule, 2'-O-methyl modifications can be added to nucleotides at position 2, positions 1 and 2, or positions 1, 2, and 3 at the 5' end of the hairpin. Also, Dicer processing of hairpins with this configuration can retain the 5' end of the sense strand intact, thus preserving the pattern of chemical modification in the post-Dicer processed duplex. Presence of a 3' overhang in this configuration can be particularly advantageous since blunt ended molecules containing the prescribed modification pattern can be further processed by Dicer in such a way that the nucleotides carrying the 2' modifications are removed. In cases where the 3' overhang is present/retained, the resulting duplex carrying the sense-modified nucleotides can have highly favorable traits with respect to silencing specificity and functionality. Examples of exemplary modification patterns are described in detail in U.S. Patent Publication No. 20050223427 and International Publication Nos. WO 2004/090105 and WO 2005/078094, the disclosures of each of which are incorporated by reference herein in their entirety.

**[0057]** shRNA may comprise sequences that were selected at random, or according to any rational design selection procedure. For example, rational design algorithms are described in International Publication No. WO 2004/045543 and U.S. Patent Publication No. 20050255487, the disclosures of which are incorporated herein by reference in their entireties. Additionally, it may be desirable to select sequences in whole or in part based on average internal stability profiles ("AISPs") or regional internal stability profiles ("RISPs") that may facilitate access or processing by cellular machinery.

**[0058]** Ribozymes are enzymatic RNA molecules capable of catalyzing specific cleavage of mRNA, thus preventing translation. (For a review, see Rossi, *Current Biology*, 4:469-471 (1994)). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage

event. The ribozyme molecules preferably include (1) one or more sequences complementary to a target mRNA, and (2) the well known catalytic sequence responsible for mRNA cleavage or a functionally equivalent sequence (see, e.g., U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety).

**[0059]** While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy target mRNAs, hammerhead ribozymes may alternatively be used. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. Preferably, the target mRNA has the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature*, 334:585-591 (1988); and PCT Application. No. WO 89/05852, the contents of which are incorporated herein by reference in its entirety.

**[0060]** Gene targeting ribozymes may contain a hybridizing region complementary to two regions of a target mRNA, each of which is at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleotides (but which need not both be the same length).

**[0061]** Hammerhead ribozyme sequences can be embedded in a stable RNA such as a transfer RNA (tRNA) to increase cleavage efficiency *in vivo* (Perriman et al., *Proc. Natl. Acad. Sci. USA*, 92:6175-79 (1995); de Feyter and Gaudron, *Methods in Molecular Biology*, Vol. 74, Chapter 43, “Expressing Ribozymes in Plants,” Turner, P. C. (ed.), Humana Press Inc., Totowa, N.J.). In particular, RNA polymerase III-mediated expression of tRNA fusion ribozymes are well known in the art (see Kawasaki et al., *Nature*, 393:284-9 (1998); Kuwabara et al., *Nature Biotechnol.*, 16:961-5 (1998); and Kuwabara et al., *Mol. Cell*, 2:617-27 (1998); Koseki et al., *J. Virol.*, 73:1868-77 (1999); Kuwabara et al., *Proc. Natl. Acad. Sci. USA*, 96:1886-91 (1999); Tanabe et al., *Nature*, 406:473-4 (2000)). There are typically a number of potential hammerhead ribozyme cleavage sites within a given target cDNA sequence. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target mRNA- to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. Furthermore, the use of any cleavage recognition site located in the target sequence encoding different portions of the target mRNA would allow the selective targeting of one or the other target genes.

**[0062]** Ribozymes for use in the inventive method also include RNA endoribonucleases (“Cech-type ribozymes”) such as the one which occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described in Zaug et

al., *Science*, 224:574-578 (1984); Zaug, et al., *Science*, 231:470-475 (1986); Zaug et al., *Nature*, 324:429-433 (1986); International Patent Publication No. WO 88/04300; and Been et al., *Cell*, 47:207-216 (1986)). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. In one embodiment, the inventive method employs those Cech-type ribozymes which target eight base-pair active site sequences that are present in a target gene or nucleic acid sequence.

**[0063]** Ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and can be chemically synthesized or produced through an expression vector. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency. Additionally, in certain embodiments, a ribozyme may be designed by first identifying a sequence portion sufficient to cause effective knockdown by RNAi. Portions of the same sequence may then be incorporated into a ribozyme.

**[0064]** Alternatively, target gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene (i.e., the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells in the body. (See generally, Helene, C., *Anticancer Drug Des.*, 6:569-84 (1991); Helene et al., *Ann. N.Y. Acad. Sci.*, 660:27-36 (1992); and Maher, L. J., *Bioassays*, 14:807-15 (1992)).

**[0065]** Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription are preferably single stranded and composed of deoxyribonucleotides. The base composition of these oligonucleotides should promote triple helix formation via Hoogsteen base pairing rules, which generally require sizable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, containing a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in CGC triplets across the three strands in the triplex.

**[0066]** Alternatively, the target sequences that can be targeted for triple helix formation may be increased by creating a so-called “switchback” nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizable stretch of either purines or pyrimidines to be present on one strand of a duplex.

**[0067]** Alternatively, DNA enzymes may be used to inhibit expression of target gene, such as the sclerostin gene. DNA enzymes incorporate some of the mechanistic features of both antisense and ribozyme technologies. DNA enzymes are designed so that they recognize a particular target nucleic acid sequence, much like an antisense oligonucleotide. They are, however, also catalytic and specifically cleave the target nucleic acid.

**[0068]** DNA enzymes include two basic types identified by Santoro and Joyce (see, for example, U.S. Patent No. 6,110,462). The 10-23 DNA enzyme comprises a loop structure which connect two arms. The two arms provide specificity by recognizing the particular target nucleic acid sequence while the loop structure provides catalytic function under physiological conditions.

**[0069]** Preferably, the unique or substantially unique sequence is a G/C rich of approximately 18 to 22 nucleotides. High G/C content helps insure a stronger interaction between the DNA enzyme and the target sequence. The specific antisense recognition sequence that will target the enzyme to the message may be divided between the two arms of the DNA enzyme.

**[0070]** Methods of making and administering DNA enzymes can be found, for example, in U.S. Patent No. 6,110,462. Additionally, one of skill in the art will recognize that, like antisense oligonucleotide, DNA enzymes can be optionally modified to improve stability and improve resistance to degradation.

**[0071]** Inhibitory oligonucleotides can be administered directly or delivered to cells by transformation or transfection via a vector, including viral vectors or plasmids, into which has been placed DNA encoding the inhibitory oligonucleotide with the appropriate regulatory sequences, including a promoter, to result in expression of the inhibitory oligonucleotide in the desired cell. Known methods include standard transient transfection, stable transfection and delivery using viruses ranging from retroviruses to adenoviruses. Delivery of nucleic acid inhibitors by replicating or replication-deficient vectors is contemplated. Expression can also be driven by either constitutive or inducible promoter systems (Paddison et al., *Methods*

*Mol. Biol.*, 265:85-100 (2004)). In other embodiments, expression may be under the control of tissue or development-specific promoters.

**[0072]** For example, vectors may be introduced by transfection using carrier compositions such as Lipofectamine 2000 (Life Technologies) or Oligofectamine (Life Technologies). Transfection efficiency may be checked using fluorescence microscopy for mammalian cell lines after co-transfection of hGFP-encoding pAD3 (Kehlenback et al., *J. Cell Biol.*, 141:863-74 (1998)).

**[0073]** The delivery route will be the one that provides the best inhibitory effect as measured according to the criteria described above. Delivery mediated by cationic liposomes, delivery by retroviral vectors and direct delivery are efficient.

**[0074]** The effectiveness of the inhibitory oligonucleotide may be assessed by any of a number of assays, including reverse transcriptase polymerase chain reaction or Northern blot analysis to determine the level of existing human sclerostin mRNA, or Western blot analysis using antibodies which recognize the human sclerostin protein, after sufficient time for turnover of the endogenous pool after new protein synthesis is repressed.

**[0075]** Activity of a particular sclerostin inhibitor, e.g., a sclerostin binding agent, for use in the inventive method may be measured in a variety of ways, including the methods described above for detecting increases in bone mineral content or bone density. The ability of a sclerostin inhibitor to modulate bone mass may be calculated from body weights or by using other methods (see Guinness-Hey, *Metab. Bone Dis. Relat. Res.*, 5:177-181 (1984)). Animals and particular animal models are used in the art for testing the effect of the pharmaceutical compositions and methods on, for example, parameters of bone loss, bone resorption, bone formation, bone strength, or bone mineralization. Examples of such models include the ovariectomized rat model (Kalu, *Bone and Mineral*, 15:175-192 (1991); Frost and Jee, *Bone and Mineral*, 18:227-236 (1992); and Jee and Yao, *J. Musculoskel. Neuron. Interact.*, 1:193-207 (2001)). The methods for measuring sclerostin binding agent activity described herein also may be used to determine the efficacy of other sclerostin inhibitors.

**[0076]** Alternatively, a sclerostin inhibitor can be selected based on its ability to modulate bone marker levels. Bone markers are products created during the bone remodeling process and are released by bone, osteoblasts, and/or osteoclasts. Fluctuations in bone resorption and/or bone formation "marker" levels imply changes in bone remodeling/modeling. The International Osteoporosis Foundation (IOF) recommends using bone markers to monitor bone density therapies (see, e.g., Delmas et al., *Osteoporos Int.*, Suppl. 6:S2-17 (2000),

incorporated herein by reference). Markers indicative of bone resorption (or osteoclast activity) include, for example, C-telopeptide (e.g., C-terminal telopeptide of type 1 collagen (CTX) or serum cross-linked C-telopeptide), N-telopeptide (N-terminal telopeptide of type 1 collagen (NTX)), deoxypyridinoline (DPD), pyridinoline, urinary hydroxyproline, galactosyl hydroxylysine, and tartrate-resistant acid phosphatase (e.g., serum tartrate-resistant acid phosphatase isoform 5b). Bone formation/mineralization markers include, but are not limited to, bone-specific alkaline phosphatase (BSAP), peptides released from N- and C-terminal extension of type I procollagen (P1NP, PICP), and osteocalcin (OstCa). Several kits are commercially-available to detect and quantify markers in clinical samples, such as urine and blood.

**[0077]** The invention is further described in the following example. The following examples serve only to illustrate the invention and are not intended to limit the scope of the invention in any way.

## EXAMPLE 1

**[0078]** This example illustrates the ability of a sclerostin inhibitor, namely an anti-sclerostin monoclonal antibody (Scl-mAb), to enhance bone healing.

**[0079]** An externally fixed femur osteotomy model (described further in Murnaghan et al., *Journal of Orthopaedic Research*, 23(3):625-631 (2005) and Connolly et al., *Journal of Orthopaedic Research*, 21:843-849 (2003)) was used to examine the effects of anti-sclerostin antibody treatment on fracture healing in mice. Eighty male CD 1 mice (9-week-old) underwent osteotomy at right femurs. A lateral incision was made through shaved skin and fascia lata from the left knee to the greater trochanter of mice under general anesthesia and aseptic conditions. The plane between the vasti and hamstrings was opened by blunt dissection to expose the femur. Four bicortical pinholes were drilled and a low-energy middiaphyseal osteotomy of the femur was performed. A custom-made drilling jig and hand saw were used ensuring exact centralization of the transverse osteotomy between the inner two pinholes. Four-pin, unilateral, single-plane, mini external fixators were applied, stabilizing the fracture. Fascia lata and skin were closed with polyglactin absorbable sutures. After the surgery, the mice were subcutaneously injected with vehicle, human parathyroid hormone-(1-34) [PTH], or an anti-sclerostin monoclonal antibody (Scl-mAb) as follows: saline vehicle (5  $\mu$ l/gram body weight), twice per week (Group 1); PTH (40  $\mu$ g/kg), five times per week for 4 weeks (Group 2); Scl-mAb (25 mg/kg), twice per week for the first week only (Group 3); and Scl-mAb (25 mg/kg), twice per week for 4 weeks (Group 4).

**[0080]** All animals were euthanized by the end of 4 weeks. Biological effects of the treatment were monitored by weekly X-ray analysis and mechanical testing. Both femurs of each subject were excised for mechanical testing. The technical challenges of femoral osteotomy model resulted in some femurs which were misaligned or incorrectly placed, and were therefore excluded from subsequent analysis. In total, 10 femurs from the vehicle-treated group (Group 1), 14 femurs from the PTH-treated group (Group 2), 15 femurs from the first week treatment group (Group 3), and 14 femurs from the 4 week treatment group (Group 4) were used in a three-point bending test to obtain bone strength.

**[0081]** Mechanical testing at the fractured femurs revealed increases in strength parameters with Scl-mAb treatment. Maximum load and stiffness were increased by 117% and 195% ( $p<0.05$ ), respectively, in the 4 week treatment group (Group 4) compared to Group 1 that received vehicle only. There was no significant difference in maximum load and stiffness among Group 1, Group 2 (the PTH treatment group), and Group 3 (receiving Scl-mAb for one week only).

**[0082]** The results described above demonstrate that inhibiting sclerostin using an anti-sclerostin antibody improved bone healing and increased bone strength of fractured sites in the externally fixed femur osteotomy model in mice.

## EXAMPLE 2

**[0083]** This example illustrates use of the inventive method to enhance bone healing *in vivo*.

**[0084]** A closed femur fracture model (described further in Bonnarens et al., *J Orthop. Res.*, 2:97–101 (1984) and Li et al., *J Bone Miner. Res.*, 18(11):2033-42 (2003)) was used to examine the effects of Scl-mAb (a sclerostin binding agent) treatment on fracture healing in rats. A standard, closed mid-diaphyseal fracture was produced in the femur in male SD rats (9-week-old). Briefly, following anesthesia, a pin (1.8 mm in diameter) was introduced into the medullary canal of the right femur, and a mid-diaphyseal fracture was created with an apparatus composed of a blunt guillotine driven by a dropped weight. After the surgery, the rats were subcutaneously injected with saline vehicle (1  $\mu$ l/gram body weight) twice a week for 2 weeks (Group 1) or 4 weeks (Group 2); Scl-mAb (25 mg/kg) twice a week for 2 weeks (Group 3); Scl-mAb (25 mg/kg) twice a week for 2 weeks (Group 4); or Scl-mAb (25 mg/kg) twice a week for 4 weeks (Group 5). Subjects in Group 3 were euthanized at the end of 2 weeks. Subjects in Groups 4 and 5 were euthanized after 4 weeks.

**[0085]** The biological response to the treatment method was monitored by weekly X-ray analysis. Both femurs were excised for mechanical testing. The technical challenges of closed femoral fracture model resulted in some femurs which were misaligned or incorrectly placed, and were therefore excluded from subsequent analysis. In total, eight femurs from vehicle-treated subjects in Group 1 and eight Scl-mAb-treated femurs from Group 3 were considered appropriate for strength testing. Eleven femurs from Group 2 subjects, 11 femurs from Group 5 subjects, and 10 femurs from Group 4 subjects (i.e., the “on/off treatment group”) were considered appropriate for strength testing.

**[0086]** Mechanical testing of the fractured femurs revealed increases in strength parameters with Scl-mAb treatment. At week 2, maximum load and stiffness were increased by 34% and 39%, respectively, in the Scl-mAb-treated group (Group 3) compared to the vehicle-treated group (Group 1). At week 4, maximum load and stiffness of fractured femurs were significantly increased by 105% and 110%, respectively, in the on/off treatment group (Group 4) compared to the matched vehicle-treated group (Group 2). Similarly, maximum load and stiffness of fractured femurs were increased by 54% and 70%, respectively, in subjects receiving Scl-mAb for 4 weeks (Group 5) compared to the vehicle-treated group (Group 2).

**[0087]** The results of this example demonstrate that administration of Scl-mAb, a sclerostin binding agent, improved bone healing and increased bone strength of fractured femurs in a closed femoral fracture rat model.

### EXAMPLE 3

**[0088]** This example illustrates use of a sclerostin inhibitor, namely an anti-sclerostin antibody, to enhance bone healing in primates.

**[0089]** Nonhuman primates provide an excellent model of fracture healing in humans due to similarities in anatomy, cortical bone remodeling, and time course of healing. The stabilized fibular osteotomy model (described further in Seeherman et al., *J Bone Joint Surg. Am.*, 86:1961-1972 (2004); Seeherman et al., *J Bone Joint Surg. Am.*, 88:144-160 (2006), and Radomsky et al., *Journal of Orthopaedic Research*, 17:607-614 (1999)) has been used in cynomolgus monkeys and baboons to investigate the effects of therapeutic agents on fracture healing. The fibular osteotomy model is minimally traumatic, consistently heals in a known timeframe, and can be performed bilaterally (on both fibulae). This model was used to examine the effects of Scl-mAb on fracture healing over a 10-week period in young male cynomolgus monkeys.

**[0090]** Forty-four male cynomolgus monkeys (aged 4-5 years) underwent bilateral fibular osteotomies. Briefly, a single transverse osteotomy was made through the fibular midshaft, and a Kirschner-wire was passed down the medullary canal from the midshaft through the distal aspect of the fibulae. The bisected fibula was realigned and the intramedullary pin was passed retrograde through the proximal half to stabilize the osteotomy. After surgery, the animals were injected subcutaneously with vehicle or Scl-mAb (25 mg/kg) biweekly for 10 weeks. The pharmacologic effects of Scl-mAb were monitored by biweekly serum biomarkers and densitometry by dual-energy X-ray absorptometry (DXA) and peripheral quantitative computed tomography (pQCT) at baseline, 6 weeks, and 10 weeks. Effects on fracture healing were assessed *ex vivo* by pQCT and torsion testing of one fibula per monkey.

**[0091]** The bone formation marker osteocalcin was significantly increased in Scl-mAb-treated subjects compared to vehicle-treated subjects throughout the study (p<0.05 at weeks 2, 4, 6, 8, and 10), with a peak increase of 50% at week 2. The bone formation marker P1NP was also significantly increased by Scl-mAb treatment (p<0.05 at weeks 2, 4, and 10), with a peak increase of 62% at week 2 compared to control animals given vehicle only. Ten weeks post-surgery, Scl-mAb treatment significantly increased the percent change in lumbar bone mineral density from baseline compared to that observed in vehicle-treated subjects (Mean±SE; Scl-mAb: 16.6±1.2%, vehicle: 4.4±0.5%). Cortical geometry at the radial diaphysis also was positively affected by Scl-mAb treatment as measured by pQCT. The percent change from baseline in periosteal perimeter was increased from 2.5% (±0.3) in vehicle-treated subjects to 4.1% (±0.4) in Scl-mAb-treated subjects.

**[0092]** In total, fibulae from 16 vehicle-treated subjects and 12 Scl-mAb-treated fibulae were considered appropriate for *ex vivo* pQCT scanning and strength testing. Peripheral QCT revealed a significant 23% increase in total callus bone mass in the Scl-mAb-treated group compared to the vehicle-treated group (p<0.05), while total callus area was non-significantly elevated by 20% (p=0.09). Calluses were thresholded by bone mineral density to separate the dense regions (“hard callus”), which reflect advanced callus maturity, from less dense regions (“soft callus”). Hard callus area and bone mineral content (BMC) were significantly increased by 26% and 29%, respectively, in Scl-mAb treated subjects compared to vehicle-treated controls, while soft callus area and associated BMC were unchanged.

**[0093]** Torsion strength testing and x-rays were subjected to a blinded review by an independent expert in biomechanics. Four additional tests from the vehicle-treatment group were excluded, resulting in 12 fibulae per group tested for strength parameters. Destructive torsion testing of the fractured fibulae revealed increases in strength parameters with Scl-

mAb treatment. Torsional stiffness was significantly increased by 48% in the Scl-mAb-treated group compared to controls ( $p<0.05$ ), while maximum torque (+32%,  $p=0.07$ ) and energy (+38%,  $p=0.12$ ) were non-significantly increased.

**[0094]** These results reveal that anti-sclerostin monoclonal antibody improved bone healing in primate osteotomy model. Biweekly subcutaneous injections of a sclerostin binding agent, Scl-mAb, increased bone formation as evidenced by increased bone formation markers, which resulted in increased bone density and improved cortical geometry in young, male monkeys. This example demonstrates the efficacy of the inventive method for increasing bone mass and torsional stiffness of the fractured fibular callus 10 weeks after osteotomy.

**[0095]** All of the references cited herein, including patents, patent applications, literature publications, and the like, are hereby incorporated in their entireties by reference.

**[0096]** While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred compounds and methods may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

## WHAT IS CLAIMED IS:

1. A method for treating a bone fracture in a subject, the method comprising administering to the subject a sclerostin binding agent in a therapeutically effective amount from about 0.5 mg/kg to about 10 mg/kg, wherein one or more administrations of the sclerostin binding agent are administered over a treatment period lasting at least two weeks and beginning within two weeks of the fracture.
2. The method of claim 1, wherein the treatment period lasts about four weeks.
3. The method of claim 1, wherein the treatment period lasts about eight weeks.
4. The method of claim 1, wherein the treatment period lasts no more than four weeks.
5. The method of claim 4, wherein the treatment period lasts two weeks.
6. The method of any one of claims 1-5, wherein the medicament comprises sclerostin binding agent in an amount from about 0.5 mg/kg to about 2.5 mg/kg.
7. The method of any one of claims 1-6, wherein the sclerostin binding agent is administered in an amount from about 1 mg/kg to about 2 mg/kg.
8. The method of any one of claims 1-8, wherein the sclerostin binding agent is administered twice a week.
9. The method of any one of claims 1-8, where the sclerostin binding agent cross-blocks the binding of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24 to sclerostin.
10. The method of any one of claims 1-9, wherein the sclerostin binding agent is cross-blocked from binding to sclerostin by at least one of antibodies Ab-A, Ab-B, Ab-C,

Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24.

11. The method of any one of claims 1-10, wherein the sclerostin binding agent is an antibody or fragment thereof that demonstrates a binding affinity for sclerostin of SEQ ID NO: 1 of less than or equal to  $1 \times 10^{-7}$  M.

12. The method of claim 11, wherein the antibody or fragment thereof comprises:  
a) CDR sequences of SEQ ID NOs:54, 55, and 56 and CDR sequences of SEQ ID NOs:51, 52, and 53; b) CDR sequences of SEQ ID NOs:60, 61, and 62 and CDR sequences of SEQ ID NOs:57, 58, and 59; c) CDR sequences of SEQ ID NOs:48, 49, and 50 and CDR sequences of SEQ ID NOs:45, 46, and 47; d) CDR sequences of SEQ ID NOs:42, 43, and 44 and CDR sequences of SEQ ID NOs:39, 40, and 41; e) CDR sequences of SEQ ID NOs:275, 276, and 277 and CDR sequences of SEQ ID NOs:287, 288, and 289; f) CDR sequences of SEQ ID NOs:278, 279, and 280 and CDR sequences of SEQ ID NOs:290, 291, and 292; g) CDR sequences of SEQ ID NOs:78, 79, and 80 and CDR sequences of SEQ ID NOs: 245, 246, and 247; h) CDR sequences of SEQ ID NOs:81, 99, and 100 and CDR sequences of SEQ ID NOs:248, 249, and 250; i) CDR sequences of SEQ ID NOs:101, 102, and 103 and CDR sequences of SEQ ID NOs:251, 252, and 253; j) CDR sequences of SEQ ID NOs:104, 105, and 106 and CDR sequences of SEQ ID NOs:254, 255, and 256; k) CDR sequences of SEQ ID NOs:107, 108, and 109 and CDR sequences of SEQ ID NOs:257, 258, and 259; l) CDR sequences of SEQ ID NOs:110, 111, and 112 and CDR sequences of SEQ ID NOs:260, 261, and 262; m) CDR sequences of SEQ ID NOs:281, 282, and 283 and CDR sequences of SEQ ID NOs:293, 294, and 295; n) CDR sequences of SEQ ID NOs:113, 114, and 115 and CDR sequences of SEQ ID NOs:263, 264, and 265; o) CDR sequences of SEQ ID NOs:284, 285, and 286 and CDR sequences of SEQ ID NOs:296, 297, and 298; p) CDR sequences of SEQ ID NOs:116, 237, and 238 and CDR sequences of SEQ ID NOs:266, 267, and 268; q) CDR sequences of SEQ ID NOs:239, 240, and 241 and CDR sequences of SEQ ID NOs:269, 270, and 271; r) CDR sequences of SEQ ID NOs:242, 243, and 244 and CDR sequences of SEQ ID NOs:272, 273, and 274; or s) CDR sequences of SEQ ID NOs:351, 352, and 353 and CDR sequences of SEQ ID NOs:358, 359, and 360.

13. The method of claim 12, wherein the antibody or fragment thereof comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1 CDR-L2 and CDR-L3 wherein (a) CDR-H1 is SEQ ID NO:245, CDR-H2 is SEQ ID NO:246, CDR-H3 is SEQ ID NO:247, CDR-L1 is SEQ ID

NO:78, CDR-L2 is SEQ ID NO:79 and CDR-L3 is SEQ ID NO:80; or (b) CDR-H1 is SEQ ID NO:269, CDR-H2 is SEQ ID NO:270, CDR-H3 is SEQ ID NO:271, CDR-L1 is SEQ ID NO:239, CDR-L2 is SEQ ID NO:240 and CDR-L3 is SEQ ID NO:241.

14. The method of any one of claims 11-13, wherein the antibody is a monoclonal antibody.
15. The method of any one of claims 11-14, wherein the antibody is a chimeric antibody.
16. The method of any one of claims 11-15, wherein the antibody is a humanized antibody.
17. The method of any one of claims 11-14, wherein the antibody is a human antibody.
18. Use of an effective amount of sclerostin binding agent in preparation of a medicament for treating a bone fracture in an amount from about 0.5 mg/kg to about 10 mg/kg, wherein one or more administrations of the medicament are administered over a treatment period lasting at least two weeks and beginning within two weeks of the fracture.
19. The use of claim 18, wherein the treatment period lasts about four weeks.
20. The use of claim 18, wherein the treatment period lasts about eight weeks.
21. The use of claim 18, wherein the treatment period lasts no more than four weeks.
22. The use of claim 21, wherein the treatment period lasts two weeks.
23. The use of any one of claims 18-22, wherein the medicament comprises sclerostin binding agent in an amount from about 0.5 mg/kg to about 2.5 mg/kg.
24. The use of any one of claims 18-23, wherein the medicament comprises sclerostin binding agent in an amount from about 1 mg/kg to about 2 mg/kg.

25. The use of any one of claims 18-24, wherein the medicament is administered twice a week.

26. The use of any one of claims 18-25, where the sclerostin binding agent cross-blocks the binding of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24 to sclerostin.

27. The use of any one of claims 18-26, wherein the sclerostin binding agent is cross-blocked from binding to sclerostin by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23, and Ab-24.

28. The use of any one of claims 18-27, wherein the sclerostin binding agent is an antibody or fragment thereof that demonstrates a binding affinity for sclerostin of SEQ ID NO: 1 of less than or equal to  $1 \times 10^{-7}$  M.

29. The use of claim 28, wherein the antibody or fragment thereof comprises: a) CDR sequences of SEQ ID NOs:54, 55, and 56 and CDR sequences of SEQ ID NOs:51, 52, and 53; b) CDR sequences of SEQ ID NOs:60, 61, and 62 and CDR sequences of SEQ ID NOs:57, 58, and 59; c) CDR sequences of SEQ ID NOs:48, 49, and 50 and CDR sequences of SEQ ID NOs:45, 46, and 47; d) CDR sequences of SEQ ID NOs:42, 43, and 44 and CDR sequences of SEQ ID NOs:39, 40, and 41; e) CDR sequences of SEQ ID NOs:275, 276, and 277 and CDR sequences of SEQ ID NOs:287, 288, and 289; f) CDR sequences of SEQ ID NOs:278, 279, and 280 and CDR sequences of SEQ ID NOs:290, 291, and 292; g) CDR sequences of SEQ ID NOs:78, 79, and 80 and CDR sequences of SEQ ID NOs: 245, 246, and 247; h) CDR sequences of SEQ ID NOs:81, 99, and 100 and CDR sequences of SEQ ID NOs:248, 249, and 250; i) CDR sequences of SEQ ID NOs:101, 102, and 103 and CDR sequences of SEQ ID NOs:251, 252, and 253; j) CDR sequences of SEQ ID NOs:104, 105, and 106 and CDR sequences of SEQ ID NOs:254, 255, and 256; k) CDR sequences of SEQ ID NOs:107, 108, and 109 and CDR sequences of SEQ ID NOs:257, 258, and 259; l) CDR sequences of SEQ ID NOs:110, 111, and 112 and CDR sequences of SEQ ID NOs:260, 261, and 262; m) CDR sequences of SEQ ID NOs:281, 282, and 283 and CDR sequences of SEQ ID NOs:293, 294, and 295; n) CDR sequences of SEQ ID NOs:113, 114, and 115 and CDR

sequences of SEQ ID NOs:263, 264, and 265; o) CDR sequences of SEQ ID NOs:284, 285, and 286 and CDR sequences of SEQ ID NOs:296, 297, and 298; p) CDR sequences of SEQ ID NOs:116, 237, and 238 and CDR sequences of SEQ ID NOs:266, 267, and 268; q) CDR sequences of SEQ ID NOs:239, 240, and 241 and CDR sequences of SEQ ID NOs:269, 270, and 271; r) CDR sequences of SEQ ID NOs:242, 243, and 244 and CDR sequences of SEQ ID NOs:272, 273, and 274; or s) CDR sequences of SEQ ID NOs:351, 352, and 353 and CDR sequences of SEQ ID NOs:358, 359, and 360.

30. The use of claim 29, wherein the antibody or fragment thereof comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1 CDR-L2 and CDR-L3 wherein (a) CDR-H1 is SEQ ID NO:245, CDR-H2 is SEQ ID NO:246, CDR-H3 is SEQ ID NO:247, CDR-L1 is SEQ ID NO:78, CDR-L2 is SEQ ID NO:79 and CDR-L3 is SEQ ID NO:80; or (b) CDR-H1 is SEQ ID NO:269, CDR-H2 is SEQ ID NO:270, CDR-H3 is SEQ ID NO:271, CDR-L1 is SEQ ID NO:239, CDR-L2 is SEQ ID NO:240 and CDR-L3 is SEQ ID NO:241.

31. The use of any one of claims 28-30, wherein the antibody is a monoclonal antibody.

32. The use of any one of claims 28-31, wherein the antibody is a chimeric antibody.

33. The use of any one of claims 28-32, wherein the antibody is a humanized antibody.

34. The use of any one of claims 28-31, wherein the antibody is a human antibody.