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(54) Title: MODULATION OF MATRIX METALLOPROTEINASE 13 (MMP-13) EXPRESSION

(57) Abstract: Disclosed herein are antisense compounds and methods for decreasing MMP-13 mRNA and protein expression. Also disclosed herein are methods for reducing osteoclastogenesis, osteolysis, osteoclasts, and TGF-beta signaling; methods for inhibiting tumor growth; methods for decreasing bone destruction and gelatinolytic activity; and method for treating or preventing metastasis of hyperproliferative diseases in an individual in need thereof.



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MODULATION OF MATRIX METALLOPROTEINASE 13 (MMP-13) EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 20110328_BIOL0129WOSEQ.txt created March 28, 2011, which is 28 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field of the Invention

Embodiments of the present invention provide methods, compounds, and compositions for inhibiting expression of MMP-13 mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate hyperproliferative diseases, including bone metastasis, such as, osteolytic breast cancer bone metastasis, osteolytic renal cell carcinoma (RCC) bone metastasis, and osteolytic prostate cancer bone metastasis.

Background of the Invention

Breast cancer is the most common cancer and the second leading cause of cancer-related death in women in the United States (Jemal, A. et al., *C.A. Cancer J. Clin.* 2009, 59: 225-49). Most complications of breast cancer are attributed to metastasis to distant organs including lymph nodes, liver, lung and bone (Boyce, B.F. et al., *Endocr. Relat. Cancer* 1999, 6: 333-47, Mundy, G.R. *Nat. Rev. Cancer* 2002, 2: 584-93). In advanced stages of the disease nearly all breast cancer patients suffer with bone metastasis. Bone metastases in breast cancer are predominantly osteolytic and also cause skeletal lesions including pathological fracture, intractable bone pain, nerve compression and hypercalcemia (Mundy, G.R. *Cancer* 1997, 80: 1546-56, Coleman, R.E. *Cancer* 1997, 80: 1588-94). These complications not only increase the risk of mortality but also cause a significant decrease in the quality of life (Mundy, G.R. *Nat. Rev. Cancer* 2002, 2: 584-93).

Extracellular Matrix (ECM) degradation, mediated by matrix metallo proteinases (MMPs), is an essential step in the growth, invasion and metastasis of malignant tumors. MMPs are a family of human zinc endopeptidases that can degrade virtually all ECM components (Birkedal-Hansen, H. *Methods Enzymol.* 1987, 144: 140-71). Apart from their ECM degradation functionality latest research in MMPs reveals their specific roles in cleaving several extracellular and membrane

associated proteins and regulating cellular signaling pathways. MMP7 promotes osteolytic bone metastasis in prostate cancer through generation of sRANKL from membrane bound RANKL (Lynch, C.C. et al., *Cancer Cell* 2005, 7: 485-96). MMP2 and MMP9 have been associated with tumor angiogenesis (Stetler-Stevenson, W.G. *Surg. Oncol. Clin. N. Am.* 2001, 10: 383-92). Expression of these proteases is also associated with poor clinical outcome in various malignancies such as bladder, breast, lung cancer and head and neck squamous cell carcinomas (Djonov, V. et al., *Int. J. Oncol.* 2002, 21: 25-30, Ruokolainen, H. et al., *Clin. Cancer Res.* 2004, 10: 3110-6).

Summary of the Invention

Provided herein are methods, compounds, and compositions for modulating expression of MMP-13 mRNA and protein. In certain embodiments, MMP-13 specific inhibitors modulate expression of MMP-13 mRNA and protein. In certain embodiments, MMP-13 specific inhibitors are nucleic acids, proteins, antibodies, or small molecules.

In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, MMP-13 mRNA levels are reduced. In certain embodiments, MMP-13 protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such diseases, disorders, and conditions are hyperproliferative diseases, disorders, and conditions. In certain embodiments such hyperproliferative diseases, disorders, and conditions include cancer as well as associated malignancies and metastases. In certain embodiments, such cancers include osteolytic breast cancer, bone metastasis, head and neck squamous cell carcinomas, breast carcinomas, bone carcinomas, laryngeal and vulvar carcinomas, non-small cell lung carcinomas, osteolytic renal cell carcinoma (RCC) bone metastasis, and osteolytic prostate cancer bone metastasis.

Such diseases, disorders, and conditions can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of a hyperproliferative disease include growing older; tobacco use; exposure to sunlight and ionizing radiation; contact with certain chemicals; infection with some viruses and bacteria; certain hormone therapies; family history of cancer; alcohol use; and certain lifestyle choices including poor diet, lack of physical activity, and/or being overweight. Certain symptoms and outcomes associated with development of a hyperproliferative disease include a thickening or lump in the breast or any other part of the body;

a new mole or a change in an existing mole; a sore that does not heal; hoarseness or a cough that does not go away; changes in bowel or bladder habits; discomfort after eating; difficulty in swallowing; unexplained weight gain or loss; unusual bleeding or discharge; fatigue; metastasis of one or more tumors throughout the body; cardiovascular complications, including, cardiac arrest and stroke; and death.

In certain embodiments, methods of treatment include administering a MMP-13 specific inhibitor to an individual in need thereof.

Detailed Description of the Invention

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

Definitions

Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents, applications, published applications and other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their

entirety.

Unless otherwise indicated, the following terms have the following meanings:

“2’-O-methoxyethyl” (also 2’-MOE and 2’-O(CH₂)₂-OCH₃) refers to an O-methoxy-ethyl modification of the 2’ position of a furosyl ring. A 2’-O-methoxyethyl modified sugar is a modified sugar.

“2’-O-methoxyethyl nucleotide” means a nucleotide comprising a 2’-O-methoxyethyl modified sugar moiety.

“5-methylcytosine” means a cytosine modified with a methyl group attached to the 5’ position. A 5-methylcytosine is a modified nucleobase.

“Active pharmaceutical agent” means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to MMP-13 is an active pharmaceutical agent.

“Active target region” or “target region” means a region to which one or more active antisense compounds is targeted. “Active antisense compounds” means antisense compounds that reduce target nucleic acid levels or protein levels.

“Administered concomitantly” refers to the co-administration of two agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both agents need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive.

“Administering” means providing a pharmaceutical agent to an individual, and includes, but is not limited to administering by a medical professional and self-administering.

“Amelioration” refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

“Animal” refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

“Antibody” refers to a molecule characterized by reacting specifically with an antigen in some way, where the antibody and the antigen are each defined in terms of the other. Antibody may

refer to a complete antibody molecule or any fragment or region thereof, such as the heavy chain, the light chain, Fab region, and Fc region.

“Antisense activity” means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

“Antisense compound” means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

“Antisense inhibition” means reduction of target nucleic acid levels or target protein levels in the presence of an antisense compound complementary to a target nucleic acid as compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

“Antisense oligonucleotide” means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

“Bicyclic sugar” means a furosyl ring modified by the bridging of two atoms. A bicyclic sugar is a modified sugar.

“Bicyclic nucleoside” means a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring.

“Cap structure” or “terminal cap moiety” means chemical modifications, which have been incorporated at either terminus of an antisense compound.

“cEt” or “constrained ethyl” means a bicyclic nucleoside having a sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-CH(CH₃)-O-2'.

“Chemically distinct region” refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

“Chimeric antisense compound” means an antisense compound that has at least two chemically distinct regions.

“Co-administration” means administration of two or more pharmaceutical agents to an individual. The two or more pharmaceutical agents may be in a single pharmaceutical composition, or may be in separate pharmaceutical compositions. Each of the two or more pharmaceutical agents

may be administered through the same or different routes of administration. Co-administration encompasses parallel or sequential administration.

“Complementarity” means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

“Contiguous nucleobases” means nucleobases immediately adjacent to each other.

“Diluent” means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, the diluent in an injected composition may be a liquid, e.g. saline solution.

“Dose” means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in one, two, or more boluses, tablets, or injections. For example, in certain embodiments where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection, therefore, two or more injections may be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week, or month.

“Effective amount” means the amount of active pharmaceutical agent sufficient to effectuate a desired physiological outcome in an individual in need of the agent. The effective amount may vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual’s medical condition, and other relevant factors.

“Fully complementary” or “100% complementary” means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

“Gapmer” means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the “gap” and the external regions may be referred to as the “wings.”

“Gap-widened” means a chimeric antisense compound having a gap segment of 12 or more contiguous 2’-deoxyribonucleosides positioned between and immediately adjacent to 5’ and 3’ wing segments having from one to six nucleosides.

“Hybridization” means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include an antisense compound and a target nucleic acid.

“Hyperproliferative disease” means a disease characterized by rapid or excessive growth and reproduction of cells. Examples of hyperproliferative diseases include cancer, *e.g.*, carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases.

“Identifying an animal at risk for hyperproliferative disease” means identifying an animal having been diagnosed with a hyperproliferative disease or identifying an animal predisposed to develop a hyperproliferative disease. Individuals predisposed to develop a hyperproliferative disease include those having one or more risk factors for hyperproliferative disease including older age; history of other hyperproliferative diseases; history of tobacco use; history of exposure to sunlight and/or ionizing radiation; prior contact with certain chemicals, especially continuous contact; past or current infection with certain viruses and bacteria; prior or current use of certain hormone therapies; genetic predisposition; alcohol use; and certain lifestyle choices including poor diet, lack of physical activity, and/or being overweight. Such identification may be accomplished by any method including evaluating an individual’s medical history and standard clinical tests or assessments.

“Immediately adjacent” means there are no intervening elements between the immediately adjacent elements.

“Inhibiting MMP-13” means reducing expression of MMP-13 mRNA and/or protein levels in the presence of a MMP-13 specific inhibitor as compared to expression of MMP-13 mRNA and/or protein levels in the absence of a MMP-13 specific inhibitor.

“Individual” means a human or non-human animal selected for treatment or therapy.

“Internucleoside linkage” refers to the chemical bond between nucleosides.

“Linked nucleosides” means adjacent nucleosides which are bonded together.

“Matrix metalloproteinase-13 nucleic acid” or “MMP-13 nucleic acid” means any nucleic acid encoding MMP-13. For example, in certain embodiments, a MMP-13 nucleic acid includes a DNA sequence encoding MMP-13, an RNA sequence transcribed from DNA encoding MMP-13 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding MMP-13. “MMP-13 mRNA” means an mRNA encoding a MMP-13 protein.

“MMP-13 specific inhibitor” refers to any agent capable of inhibiting the expression of MMP-13 mRNA and/or MMP-13 protein with few to no off-target effects. MMP-13 specific

inhibitors include, but are not limited to, nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of MMP-13 mRNA and/or MMP-13 protein. In certain embodiments, by specifically modulating MMP-13 mRNA expression and/or MMP-13 protein expression, MMP-13 specific inhibitors affect other downstream proteins and molecules. For example, in certain embodiments, an MMP-13 specific inhibitor affects RANKL, MMP9, and TGF beta mRNA and protein expression. In certain embodiments, MMP-13 specific inhibitors affect certain molecular processes or pathways in an animal. For example, in certain embodiments, an MMP-13 specific inhibitor affects osteoclastogenesis, osteolysis, formation of osteoclasts, TGF-beta signaling, tumor growth, rate of bone destruction, and gelatinolytic activity.

“Mismatch” or “non-complementary nucleobase” refers to the case when a nucleobase of a first nucleic acid is not capable of pairing with the corresponding nucleobase of a second or target nucleic acid.

“Modified internucleoside linkage” refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

“Modified nucleobase” refers to any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An “unmodified nucleobase” means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U).

“Modified nucleotide” means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase. A “modified nucleoside” means a nucleoside having, independently, a modified sugar moiety or modified nucleobase.

“Modified oligonucleotide” means an oligonucleotide comprising a modified internucleoside linkage, a modified sugar, or a modified nucleobase.

“Modified sugar” refers to a substitution or change from a natural sugar.

“Motif” means the pattern of chemically distinct regions in an antisense compound.

“Naturally occurring internucleoside linkage” means a 3' to 5' phosphodiester linkage.

“Natural sugar moiety” means a sugar found in DNA (2'-H) or RNA (2'-OH).

“Nucleic acid” refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA).

“Nucleobase” means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

“Nucleobase sequence” means the order of contiguous nucleobases independent of any sugar, linkage, or nucleobase modification.

“Nucleoside” means a nucleobase linked to a sugar.

“Nucleoside mimetic” includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics, *e.g.*, non furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system.

“Nucleotide” means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

“Off-target effect” refers to an unwanted or deleterious biological effect associated with modulation of RNA or protein expression of a gene other than the intended target nucleic acid.

“Oligomeric compound” or “oligomer” means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

“Oligonucleotide” means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

“Parenteral administration” means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, *e.g.*, intrathecal or intracerebroventricular administration.

“Peptide” means a molecule formed by linking at least two amino acids by amide bonds. Peptide refers to polypeptides and proteins.

“Pharmaceutical composition” means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition may comprise one or more active pharmaceutical agents and a sterile aqueous solution.

“Pharmaceutically acceptable salts” means physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

“Phosphorothioate linkage” means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage (P=S) is a modified internucleoside linkage.

“Portion” means a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

“Prevent” refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to indefinitely. Prevent also means reducing risk of developing a disease, disorder, or condition.

“Prodrug” means a therapeutic agent that is prepared in an inactive form that is converted to an active form within the body or cells thereof by the action of endogenous enzymes or other chemicals or conditions.

“Side effects” means physiological responses attributable to a treatment other than the desired effects. In certain embodiments, side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

“Single-stranded oligonucleotide” means an oligonucleotide which is not hybridized to a complementary strand.

“Specifically hybridizable” refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays and therapeutic treatments.

“Targeting” or “targeted” means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

“Target nucleic acid,” “target RNA,” “target mRNA,” and “target RNA transcript” all refer to a nucleic acid capable of being targeted by antisense compounds.

“Target segment” means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. “5’ target site” refers to the 5’-most nucleotide of a target segment. “3’ target site” refers to the 3’-most nucleotide of a target segment.

“Therapeutically effective amount” means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

“Treat” refers to administering a pharmaceutical composition to effect an alteration or improvement of a disease, disorder, or condition.

“Unmodified nucleotide” means a nucleotide composed of naturally occurring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. β -D-ribonucleosides) or a DNA nucleotide (i.e. β -D-deoxyribonucleoside).

Certain Embodiments

Embodiments of the present invention provide methods, compounds, and compositions for inhibiting MMP-13 mRNA or protein expression.

Embodiments of the present invention provide methods, compounds, and compositions for inhibiting RANKL, MMP9, or TGF beta mRNA or protein expression.

Embodiments of the present invention provide methods for reducing RANKL:OPG ratio.

Embodiments of the present invention provide methods for reducing osteoclastogenesis, osteolysis, number of osteoclasts, and TGF-beta signaling. Embodiments of the present invention provide methods for reducing tumor induced osteolysis.

Embodiments of the present invention provide methods for preventing tumor growth and tumor volume. Embodiments of the present invention provide methods for reducing tumor growth and tumor volume.

Embodiments of the present invention provide methods for decreasing bone destruction and gelatinolytic activity. Embodiments of the present invention provide methods for decreasing bone destruction index (BDI).

Embodiments of the present invention provide methods for reducing active MMP-9:pro-MMP-9 ratio.

Embodiments of the present invention provide methods for reducing pSmad2 positive index.

Embodiments of the present invention provide methods for reducing TGF-beta signaling.

Embodiments of the present invention provide methods, compounds, and compositions for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with MMP-13 in an individual in need thereof. Also contemplated are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with MMP-13. MMP-13 associated diseases, disorders, and conditions include hyperproliferative diseases, *e.g.*, cancer, carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases.

Embodiments of the present invention provide a MMP-13 specific inhibitor for use in treating, preventing, or ameliorating a MMP-13 associated disease. In certain embodiments, MMP-13 specific inhibitors are nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of MMP-13 mRNA and/or MMP-13 protein.

Embodiments of the present invention provide a MMP-13 specific inhibitor as described herein for use in treating or preventing a hyperproliferative disease.

Embodiments of the present invention provide a MMP-13 specific inhibitor as described herein for use in treating or preventing breast cancer, bone cancer, head and neck squamous cell carcinomas, laryngeal and vulvar carcinomas, non-small cell lung carcinomas, prostate cancer, and renal cell carcinoma (RCC).

Embodiments of the present invention provide a MMP-13 specific inhibitor as described herein for use in treating or preventing cancer from metastasizing to bone.

Embodiments of the present invention provide a MMP-13 specific inhibitor as described herein for reducing osteoclastogenesis, reducing osteolysis, reducing tumor induced osteolysis, inhibiting tumor growth, inhibiting tumor volume, decreasing bone destruction, decreasing the bone destruction index, reducing osteoclast number, decreasing gelatinolytic activity, reducing TGF-beta signaling, reducing the pSmad2 positive index, reducing the active MMP-9:pro-MMP-9 ratio, reducing the RANKL:OPG ratio, or reducing risk for osteoclastogenesis.

Embodiments of the present invention provide a MMP-13 specific inhibitor as described herein for reducing expression of MMP-13, MMP-9, RANKL, or TGF-beta mRNA or protein expression in cells or tissue.

Embodiments of the present invention provide a composition comprising a MMP-13 specific inhibitor as described herein for reducing osteoclastogenesis, reducing osteolysis, reducing tumor induced osteolysis, inhibiting tumor growth, inhibiting tumor volume, decreasing bone destruction, decreasing the bone destruction index, reducing osteoclast number, decreasing gelatinolytic activity, reducing TGF-beta signaling, reducing the pSmad2 positive index, reducing the active MMP-9:pro-MMP-9 ratio, reducing the RANKL:OPG ratio, or reducing risk for osteoclastogenesis.

In certain embodiments of the present invention, MMP-13 specific inhibitors are peptides or proteins, such as, but not limited to, RXP03 (Dive, V. *et al.*, *Int. J. Cancer* 2004, 113: 775-781), ITZ-1 (Kimura, H. *et al.*, *Chem. Biol.* 2010, 17: 18-27), interferon- γ (Ala-aho, R. *et al.*, *Oncogene* 2000, 19: 248-257), 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) (Cevik, C. *et al.*, *J. Cardiovasc. Med. (Hagerstown)*. 2008, 9: 1274-8), recombinant Ac-TMP-2 protein (Zhan, B., *et al.*, *Mol. Biochem. Parasitol.* 2008, 162: 142-8), and insulin like growth factor-1 (IGF-1) (Zhang, M. *et al.*, *Osteoarthritis Cartilage*. 2009, 17: 100-6).

In certain embodiments of the present invention, MMP-13 specific inhibitors are antibodies, such as, but not limited to, rabbit anti-human MMP-13 (Calbiochem) and goat-anti-human-MMP-13 (Santa Cruz Biotechnologies).

In certain embodiments of the present invention, MMP-13 specific inhibitors are small molecules, such as, but not limited to, CL-82198 (Hernández Ríos, M. *et al.*, *J. Clin. Periodontol.* 2009, 36: 1011-7), RS102,481 (Billinghurst, R.C. *et al.*, *Arthritis Rheum.* 2000, **43**: 664 – 672), triolein (Kim, E.J. *et al.*, *J. Dermatol. Sci.* 2010, 57: 19-26), CL-82198 (Hernández Ríos, M. *et al.*, *J. Clin. Periodontol.* 2009, 36: 1011-7) *N*-*O*-Isopropyl Sulfonamido-Based Hydroxamates (Nuti, E. *et al.*, *J. Med. Chem.* 2009, 52: 4757-73), ITZ-1 (Kimura, H. *et al.*, *J. Pharmacol. Sci.* 2009, 110: 201-11), Praziquantel (Pinlaor, S. *et al.*, *Acta Trop.* 2009, 111: 181-91), 24f (Monovich, L.G. *et al.*, *J. Med. Chem.* 2009, 52: 3523-38), GM6001 (Hancox, R.A. *et al.*, *Breast Cancer Res.* 2009, 11: R24), extracts of *Euphorbia hirta* (Lee, K.H. *et al.*, *Pathol.* 2008, 30: 95-102), KHBj-9B (Huh, J.E. *et al.*, *Int. Immunopharmacol.* 2009, 9: 230-40), ilomastat (Attur, M. *et al.*, *J. Immunol.* 2008, 181: 5082-8), *N*-hydroxy-2-[(phenylsulfonyl)amino]acetamide (Fernandez, M. *et al.*, *Chem. Biol. Drug Des.* 2008, 72: 65-78), and taurine chloramines (Kim, K.S. *et al.*, *Arthritis Res. Ther.* 2007, 9: R80).

Embodiments of the present invention provide a MMP-13 specific inhibitor, as described herein, for use in treating, preventing, or ameliorating hyperproliferative diseases, *e.g.*, cancer, carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases. Examples of such hyperproliferative diseases include breast cancer and carcinomas,

bone cancer and carcinomas, head and neck squamous cell carcinomas, laryngeal and vulvar carcinomas, non-small cell lung carcinomas, prostate cancer, and renal cell carcinoma (RCC). Examples of such metastases include osteolytic breast cancer bone metastasis, osteolytic renal cell carcinoma (RCC) bone metastasis, and osteolytic prostate bone metastasis.

Embodiments of the present invention provide antisense compounds targeted to a MMP-13 nucleic acid. In certain embodiments, the MMP-13 nucleic acid is any of the sequences set forth in GENBANK Accession No. M60616.1 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. BE127506.1 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. AW914210.1 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. NM_002427.2 (incorporated herein as SEQ ID NO: 4, GENBANK Accession No. NM_008607.1 (incorporated herein as SEQ ID NO: 7).

Embodiments of the present invention provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85.

In certain embodiments, the compound consists of a single-stranded modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is 100% complementary to a nucleobase sequence of SEQ ID NO: 4.

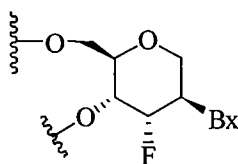
In certain embodiments, at least one internucleoside linkage is a modified internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, at least one nucleoside comprises a modified sugar.

In certain embodiments, at least one modified sugar is a bicyclic sugar. In certain embodiments, each of the at least one bicyclic sugar comprises a 4'- $(\text{CH}_2)_n$ -O-2' bridge, wherein n is 1 or 2. In certain embodiments, each of the at least one bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.

In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group.

In certain embodiments, at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring. In certain embodiments, each of the at least one tetrahydropyran modified nucleoside has the structure:



wherein Bx is an optionally protected heterocyclic base moiety.

In certain embodiments, at least one nucleoside comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide comprises a gap segment consisting of linked deoxynucleosides, a 5' wing segment consisting of linked nucleosides, a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In certain embodiments, the modified oligonucleotide comprises a gap segment consisting of ten linked deoxynucleosides, a 5' wing segment consisting of five linked nucleosides, a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, wherein each internucleoside linkage is a phosphorothioate linkage, and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides.

In certain embodiments, the present invention provides a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of reducing osteoclastogenesis in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing osteolysis in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of

a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing tumor induced osteolysis in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of inhibiting tumor growth in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of inhibiting tumor volume in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of decreasing bone destruction in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of decreasing bone destruction index in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing osteoclast number in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of decreasing gelatinolytic activity in an animal in need thereof, comprising administering to the animal a therapeutically

effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing TGF-beta signaling in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing pSmad2 positive index in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing active MMP-9:pro-MMP-9 ratio in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing RANKL:OPG ratio in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method comprising reducing the risk for osteoclastogenesis in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method comprising treating cancer in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

In certain embodiments, the present invention provides a method of reducing osteoclastogenesis in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of reducing osteolysis in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of inhibiting tumor growth in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of decreasing bone destruction in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of reducing osteoclasts in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of decreasing gelatinolytic activity in an animal in need thereof, comprising administering to the animal a therapeutically

effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of reducing TGF-beta signaling in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method comprising reducing the risk for osteoclastogenesis in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method comprising treating cancer in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method comprising treating renal cell carcinoma in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method comprising treating breast cancer in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous

nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method comprising treating non small cell carcinoma in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method comprising treating prostate cancer in an animal in need thereof, comprising administering to the animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present invention provides a method of inhibiting expression of MMP13 in cells or tissues comprising contacting the cells or tissue with a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85.

In certain embodiments, the present invention provides a method of inhibiting expression of RANKL in cells or tissues comprising contacting the cells or tissue with a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85.

In certain embodiments, the present invention provides a method of inhibiting expression of MMP-9 in cells or tissues comprising contacting the cells or tissue with a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85.

In certain embodiments, the present invention provides a method of inhibiting expression of TGF beta in cells or tissues comprising contacting the cells or tissue with a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase

sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85.

In certain embodiments, the cells or tissues are human cells or tissues.

In certain embodiments, the cells or tissues are at the tumor bone interface.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 230 to 249 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 338 to 357 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 548 to 567 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 627 to 653 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 719 to 738 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion

of nucleobases 812 to 846 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 929 to 953 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1004 to 1023 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1317 to 1341 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1367 to 1386 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1545 to 1564 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

In certain embodiments, the present invention provides a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1594 to 1623 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

Antisense Compounds

Oligomeric compounds include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be “antisense” to a target nucleic acid, meaning that it is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted. In certain such embodiments, an antisense oligonucleotide has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, an antisense compound targeted to a MMP-13 nucleic acid is 12 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits. In other embodiments, the antisense compound is 8 to 80, 12 to 50, 15 to 30, 18 to 24, 19 to 22, or 20 linked subunits. In certain such embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleotides.

In certain embodiments antisense oligonucleotides targeted to a MMP-13 nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a MMP-13 nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more

additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

Gautschi et al. (J. Natl. Cancer Inst. 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL *in vitro* and *in vivo*. Furthermore, this oligonucleotide demonstrated potent anti-tumor activity *in vivo*.

Maher and Dolnick (Nuc. Acid. Res. 16:3341-3358, 1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to a MMP-13 nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding

affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE, and 2'-O-CH₃, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH₂)_n-O-2' bridge, where n=1 or n=2). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap segment is positioned immediately adjacent each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing segment and gap segment, or the gap segment and the 3' wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleotides. Thus, gapmers of the present invention include, but are not limited to, for example 5-10-5, 4-8-4, 4-12-3, 4-12-4, 3-14-3, 2-13-5, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1, 2-8-2, 5-8-5, or 6-8-6.

In certain embodiments, the antisense compound has a "wingmer" motif, having a wing-gap or gap-wing configuration, i.e. an X-Y or Y-Z configuration as described above for the gapmer configuration. Thus, wingmer configurations of the present invention include, but are not limited to, for example 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, 5-13, 5-8, or 6-8.

In certain embodiments, antisense compounds targeted to a MMP-13 nucleic acid possess a 5-10-5 gapmer motif.

In certain embodiments, an antisense compound targeted to a MMP-13 nucleic acid has a gap-widened motif.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode MMP-13 include, without limitation, the following: GENBANK Accession No. M60616.1, incorporated herein as SEQ ID NO: 1; BE127506.1, incorporated herein as SEQ ID NO: 2; GENBANK Accession No. AW914210.1, incorporated herein as SEQ ID NO: 3; and GENBANK Accession No. NM_002427.2, incorporated herein as SEQ ID NO: 4; and GENBANK Accession No. NM_008607.1, incorporated herein as SEQ ID NO: 7.

It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for MMP-13 can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels. In certain embodiments, the desired effect is reduction of levels of protein encoded by the target nucleic acid or a phenotypic change associated with the target nucleic acid.

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300

nucleotides. In certain embodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifically exclude a certain structurally defined region such as the start codon or stop codon.

The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in MMP-13 mRNA levels are indicative of inhibition of MMP-13 expression. Reductions in levels of a MMP-13 protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of MMP-13 expression. For example, reduced cellular growth, reduced tumor growth, and reduced tumor volume can be indicative of inhibition of MMP-13 expression. In another example, reduced osteolysis can be indicative of inhibition of MMP-13 expression. In certain embodiments, the reduced osteolysis can be tumor induced osteolysis. In another example, reduced expression of RANKL, MMP-9, TGF beta mRNA and protein can be indicative of inhibition of MMP-13 expression. In another example, reduced RANKL:OPG ratio can be indicative of inhibition of MMP-13 expression. In another example, reduced bone destruction and bone destruction index can be indicative of MMP-13 expression. In another example, reduced osteoclast number can be indicative of MMP-13 expression. In another example, reduced active MMP-9:pro-MMP-9 ratio can be indicative of MMP-13 expression. In another example, reduced pSmad2 positive index can be indicative of MMP-13 expression. In another example, reduced TGF-beta signaling can be indicative of MMP-13 expression. In another

example, amelioration of symptoms associated with cancer can be indicative of inhibition of MMP-13 expression. In another example, reduction of cancer markers can be indicative of inhibition of MMP-13 expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a MMP-13 nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a MMP-13 nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a MMP-13 nucleic acid).

Non-complementary nucleobases between an antisense compound and a MMP-13 nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a MMP-13 nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a MMP-13 nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having four noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403 410; Zhang and Madden, *Genome Res.*, 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, 1981, 2, 482 489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a MMP-13 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, “fully complementary” means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be “fully complementary” to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully

complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a MMP-13 nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a MMP-13 nucleic acid, or specified portion thereof.

The antisense compounds provided herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of

thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, a portion of the antisense oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular

uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a MMP-13 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds of the invention can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring moiety. Examples of chemically modified ribofuranose rings include, without limitation, addition of substituent groups (including 5' and 2' substituent groups); bridging of non-

geminal ring atoms to form bicyclic nucleic acids (BNA); replacement of the ribosyl ring oxygen atom with S, N(R), or C(R1)(R)2 (R = H, C₁-C₁₂ alkyl or a protecting group); and combinations thereof. Examples of chemically modified sugars include, 2'-F-5'-methyl substituted nucleoside (*see*, PCT International Application WO 2008/101157, published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides), replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (*see*, published U.S. Patent Application US2005/0130923, published on June 16, 2005), or, alternatively, 5'-substitution of a BNA (*see*, PCT International Application WO 2007/134181, published on 11/22/07, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include, without limitation, nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH₃, and 2'-O(CH₂)₂OCH₃ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, OCF₃, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(R_m)(R_n), and O-CH₂-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include, without limitation, nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises a 4' to 2' bicyclic nucleoside. Examples of such 4' to 2' bicyclic nucleosides, include, but are not limited to, one of the formulae: 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' and 4'-CH(CH₂OCH₃)-O-2', and analogs thereof (*see*, U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2', and analogs thereof (*see*, published PCT International Application WO2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2', and analogs thereof (*see*, published PCT International Application WO2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (*see*, published U.S. Patent Application US2004/0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (*see*, U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (*see*, Chattopadhyaya, *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2', and analogs thereof (*see*, published PCT International Application WO 2008/154401, published on December 8, 2008). Also *see*, for example: Singh *et al.*, *Chem. Commun.*, 1998, 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630; Wahlestedt *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Kumar *et al.*,

Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava et al., *J. Am. Chem. Soc.*, 129(26) 8362-8379 (Jul. 4, 2007); Elayadi et al., *Curr. Opinion Invens. Drugs*, 2001, 2, 558-561; Braasch et al., *Chem. Biol.*, 2001, 8, 1-7; Orum et al., *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; U.S. Patent Nos U.S. 6,670,461, 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 7,399,845; published PCT International applications WO 2004/106356, WO 94/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; and U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Application Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the pentofuranosyl sugar moiety wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from $-[C(R_a)(R_b)]_n-$, $-C(R_a)=C(R_b)-$, $-C(R_a)=N-$, $-C(=NR_a)-$, $-C(=O)-$, $-C(=S)-$, $-O-$, $-Si(R_a)_2-$, $-S(=O)_x-$, and $-N(R_a)-$;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

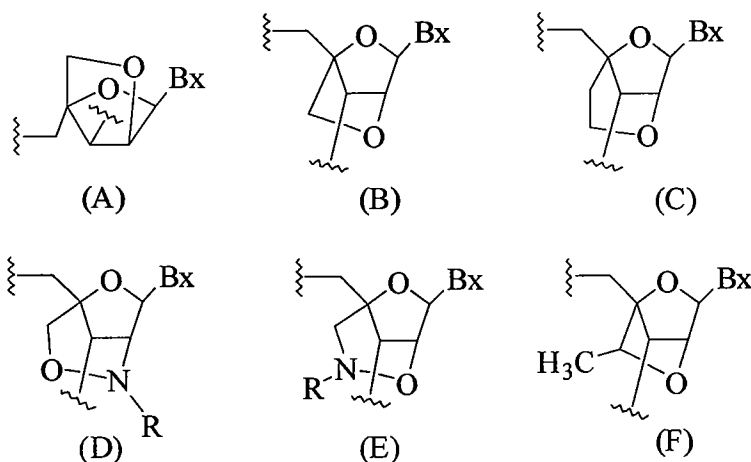
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5 - C_7 alicyclic radical, substituted C_5 - C_7 alicyclic radical, halogen, OJ_1 , NJ_1J_2 , SJ_1 , N_3 , $COOJ_1$, acyl ($C(=O)-H$), substituted acyl, CN, sulfonyl ($S(=O)_2J_1$), or sulfoxyl ($S(=O)-J_1$); and

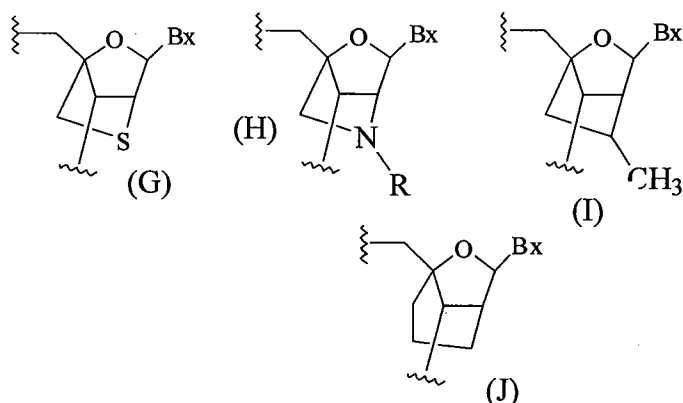
each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)-H$), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted C_1 - C_{12} aminoalkyl, or a protecting group.

In certain embodiments, the bridge of a bicyclic sugar moiety is, $-[C(R_a)(R_b)]_n-$, $-[C(R_a)(R_b)]_n-O-$, $-C(R_aR_b)-N(R)-O-$ or, $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is $4'-CH_2-2'$, $4'-(CH_2)_2-2'$, $4'-(CH_2)_3-2'$, $4'-CH_2-O-2'$, $4'-(CH_2)_2-O-2'$, $4'-CH_2-O-N(R)-2'$, and $4'-CH_2-N(R)-O-2'$, wherein each R is, independently, H, a protecting group, or C_1-C_{12} alkyl.

In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a $4'-2'$ methylene-oxy bridge, may be in the α -L configuration or in the β -D configuration. Previously, α -L-methyleneoxy ($4'-CH_2-O-2'$) BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden *et al.*, *Nucleic Acids Research*, 2003, 21, 6365-6372).

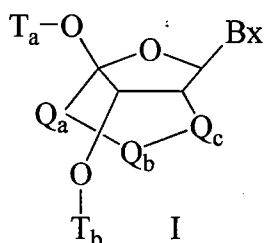
In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α -L-Methyleneoxy ($4'-CH_2-O-2'$) BNA, (B) β -D-Methyleneoxy ($4'-CH_2-O-2'$) BNA, (C) Ethyleneoxy ($4'-(CH_2)_2-O-2'$) BNA, (D) Aminooxy ($4'-CH_2-O-N(R)-2'$) BNA, (E) Oxyamino ($4'-CH_2-N(R)-O-2'$) BNA, (F) Methyl(methyleneoxy) ($4'-CH(CH_3)-O-2'$) BNA, (G) methylene-thio ($4'-CH_2-S-2'$) BNA, (H) methylene-amino ($4'-CH_2-N(R)-2'$) BNA, (I) methyl carbocyclic ($4'-CH_2-CH(CH_3)-2'$) BNA, and (J) propylene carbocyclic ($4'-(CH_2)_3-2'$) BNA as depicted below.





wherein Bx is the base moiety and R is, independently, H, a protecting group or C₁-C₁₂ alkyl.

In certain embodiments, bicyclic nucleoside having Formula I:



wherein:

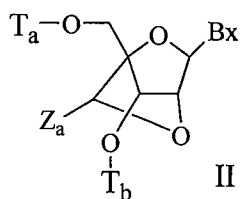
Bx is a heterocyclic base moiety;

-Q_a-Q_b-Q_c- is -CH₂-N(R_c)-CH₂-, -C(=O)-N(R_c)-CH₂-, -CH₂-O-N(R_c)-, -CH₂-N(R_c)-O-, or -N(R_c)-O-CH₂;

R_c is C₁-C₁₂ alkyl or an amino protecting group; and

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium.

In certain embodiments, bicyclic nucleoside having Formula II:



wherein:

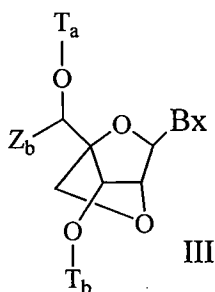
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

Z_a is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, substituted C_1 - C_6 alkyl, substituted C_2 - C_6 alkenyl, substituted C_2 - C_6 alkynyl, acyl, substituted acyl, substituted amide, thiol, or substituted thio.

In one embodiment, each of the substituted groups is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c , NJ_cJ_d , SJ_c , N_3 , $OC(=X)J_c$, and $NJ_eC(=X)NJ_cJ_d$, wherein each J_c , J_d , and J_e is, independently, H, C_1 - C_6 alkyl, or substituted C_1 - C_6 alkyl and X is O or NJ_c .

In certain embodiments, bicyclic nucleoside having Formula III:



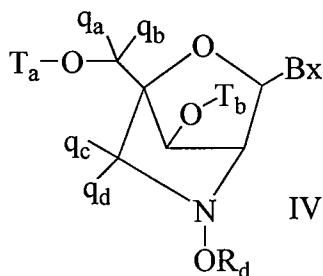
wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

Z_b is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, substituted C_1 - C_6 alkyl, substituted C_2 - C_6 alkenyl, substituted C_2 - C_6 alkynyl, or substituted acyl ($C(=O)-$).

In certain embodiments, bicyclic nucleoside having Formula IV:



wherein:

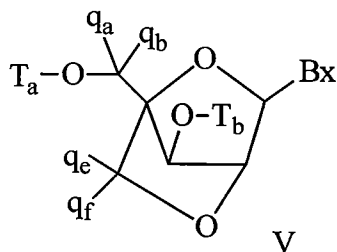
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

R_d is C_1 - C_6 alkyl, substituted C_1 - C_6 alkyl, C_2 - C_6 alkenyl, substituted C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, or substituted C_2 - C_6 alkynyl;

each q_a , q_b , q_c and q_d is, independently, H, halogen, C_1 - C_6 alkyl, substituted C_1 - C_6 alkyl, C_2 - C_6 alkenyl, substituted C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, or substituted C_2 - C_6 alkynyl, C_1 - C_6 alkoxy, substituted C_1 - C_6 alkoxy, acyl, substituted acyl, C_1 - C_6 aminoalkyl, or substituted C_1 - C_6 aminoalkyl;

In certain embodiments, bicyclic nucleoside having Formula V:



wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

q_a , q_b , q_e and q_f are each, independently, hydrogen, halogen, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_1 - C_{12} alkoxy, substituted C_1 - C_{12} alkoxy, OJ_j , SJ_j , SOJ_j , SO_2J_j , NJ_jJ_k , N_3 , CN , $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O-C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

or q_e and q_f together are $=C(q_g)(q_h)$;

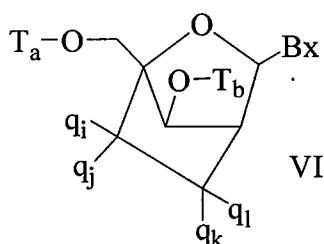
q_g and q_h are each, independently, H, halogen, C_1 - C_{12} alkyl, or substituted C_1 - C_{12} alkyl.

The synthesis and preparation of the methyleneoxy (4'- CH_2 -O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine, and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (*see, e.g.*, Koshkin et al., *Tetrahedron*, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogues of methyleneoxy (4'- CH_2 -O-2') BNA, methyleneoxy (4'- CH_2 -O-2') BNA, and 2'-thio-BNAs, have also been prepared (*see, e.g.*, Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (*see, e.g.*, Wengel et al., WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel conformationally restricted

high-affinity oligonucleotide analog, has been described in the art (*see, e.g.*, Singh et al., *J. Org. Chem.*, 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methyldamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

In certain embodiments, bicyclic nucleoside having Formula VI:



wherein:

Bx is a heterocyclic base moiety;

Ta and Tb are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

each qi, qj, qk and ql is, independently, H, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k, or N(H)C(=S)NJ_jJ_k; and

qi and qj or ql and qk together are =C(q_g)(q_h), wherein q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl, or substituted C₁-C₁₂ alkyl.

One carbocyclic bicyclic nucleoside having a 4'-(CH₂)₃-2' bridge and the alkenyl analog, bridge 4'-CH=CH-CH₂-2', have been described (*see, e.g.*, Freier et al., *Nucleic Acids Research*, 1997, 25(22), 4429-4443 and Albaek et al., *J. Org. Chem.*, 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.*, Srivastava et al., *J. Am. Chem. Soc.* 2007, 129(26), 8362-8379).

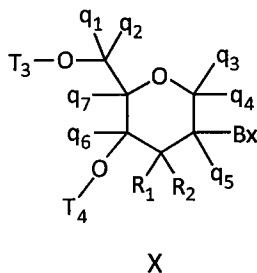
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting the 2' carbon atom and the 4' carbon atom.

As used herein, "monocyclic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'-substituent groups can also be selected from: C_1 - C_{12} alkyl; substituted alkyl; alkenyl; alkynyl; alkaryl; aralkyl; O-alkaryl or O-aralkyl; SH; SCH_3 ; OCN; Cl; Br; CN; CF_3 ; OCF_3 ; $SOCH_3$; SO_2CH_3 ; ONO_2 ; NO_2 ; N_3 ; NH_2 ; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving pharmacokinetic properties; and a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modified nucleosides comprise a 2'-MOE side chain (*see, e.g.*, Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'-O-methyl, O-propyl, and O-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (*see, e.g.*, Martin, P., *Helv. Chim. Acta*, 1995, 78, 486-504; Altmann et al., *Chimia*, 1996, 50, 168-176; Altmann et al., *Biochem. Soc. Trans.*, 1996, 24, 630-637; and Altmann et al., *Nucleosides Nucleotides*, 1997, 16, 917-926).

As used herein, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (*see* Leumann, CJ. *Bioorg. & Med. Chem.* (2002) 10:841-854), fluoro HNA (F-HNA), or those compounds having Formula X:

Formula X:



wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula X:

Bx is a heterocyclic base moiety;

T₃ and T₄ are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T₃ and T₄ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl; and

one of R₁ and R₂ is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ₃C(=X)NJ₁J₂, and CN, wherein X is O, S, or NJ₁, and each J₁, J₂, and J₃ is, independently, H or C₁-C₆ alkyl.

In certain embodiments, the modified THP nucleosides of Formula X are provided wherein q_m, q_n, q_p, q_r, q_s, q_t, and q_u are each H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t, and q_u is other than H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t and q_u is methyl. In certain embodiments, THP nucleosides of Formula X are provided wherein one of R₁ and R₂ is F. In certain embodiments, R₁ is fluoro and R₂ is H, R₁ is methoxy and R₂ is H, and R₁ is methoxyethoxy and R₂ is H.

As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring and nucleosides with non-bridging 2' substituents, such as allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, -OCF₃, O-(CH₂)₂-O-CH₃, 2'-O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R_m)(R_n), or O-CH₂-C(=O)-N(R_m)(R_n), where each R_m and

R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl. 2'-modified nucleosides may further comprise other modifications, for example, at other positions of the sugar and/or at the nucleobase.

As used herein, "2'-F" refers to a nucleoside comprising a sugar comprising a fluoro group at the 2' position.

As used herein, "2'-OMe" or "2'-OCH₃" or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an -OCH₃ group at the 2' position of the sugar ring.

As used herein, "MOE" or "2'-MOE" or "2'-OCH₂CH₂OCH₃" or "2'-O-methoxyethyl" each refers to a nucleoside comprising a sugar comprising a -OCH₂CH₂OCH₃ group at the 2' position of the sugar ring.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (*see, e.g.,* review article: Leumann, J. C, *Bioorganic & Medicinal Chemistry*, **2002**, *10*, 841-854).

Such ring systems can undergo various additional substitutions to enhance activity.

Methods for the preparations of modified sugars are well known to those skilled in the art.

In nucleotides having modified sugar moieties, the nucleobase moieties (natural, modified, or a combination thereof) are maintained for hybridization with an appropriate nucleic acid target.

In certain embodiments, antisense compounds comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a cEt. In certain embodiments, the cEt modified nucleotides are arranged throughout the wings of a gapmer motif.

Compositions and Methods for Formulating Pharmaceutical Compositions

Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of

criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

An antisense compound targeted to a MMP-13 nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a MMP-13 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

Conjugated Antisense Compounds

Antisense compounds may be covalently linked to one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. Typical conjugate groups include cholesterol moieties and lipid moieties. Additional conjugate groups include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the

5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of MMP-13 nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commercial vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (*e.g.* Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, mammary adenocarcinoma cell lines, including, 4T1 having a high metastatic potential, C166 having a moderate metastatic potential, and C166M2 having a low metastatic potential; HepG2 cells; Hep3B cells; TM-3; and primary hepatocytes.

In vitro testing of antisense oligonucleotides

Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

Cells may be treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN (Invitrogen, Carlsbad, CA). Antisense oligonucleotides may be mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE in OPTI-MEM 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a LIPOFECTAMINE concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

Cells are treated with antisense oligonucleotides by routine methods. Cells may be harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein. In general, when treatments are performed in multiple replicates, the data are presented as the average of the replicate treatments.

The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. RNA is prepared using methods well known in the art, for example, using the TRIZOL Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocols.

Analysis of inhibition of target levels or expression

Inhibition of levels or expression of a MMP-13 nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitative real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Methods of quantitative real-time PCR are well known in the art.

Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT and real-time PCR reagents may be obtained from Invitrogen (Carlsbad, CA). RT real-time-PCR reactions are carried out by methods well known to those skilled in the art.

Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN RNA quantification reagent (Invitrogen, Inc. Eugene, OR). Methods of RNA quantification by RIBOGREEN are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN fluorescence.

Probes and primers are designed to hybridize to a MMP-13 nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of MMP-13 nucleic acids can be assessed by measuring MMP-13 protein levels. Protein levels of MMP-13 can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of mouse, rat, monkey, and human MMP-13 are commercially available.

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of MMP-13 and produce phenotypic changes, such as, reduced cellular growth; reduced osteolysis, including tumor induced osteolysis; amelioration of symptoms associated with cancer; and reduction of cancer markers. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, and subcutaneous. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from liver tissue and changes in MMP-13 nucleic acid expression are measured. Changes in MMP-13 protein levels are also measured.

Certain Indications

In certain embodiments, the invention provides methods, compounds, and compositions of treating an individual comprising administering one or more pharmaceutical compositions of the present invention. In certain embodiments, the individual has a hyperproliferative disease. In certain embodiments, the hyperproliferative disease is cancer, e.g., carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases. In certain embodiments, the type of cancer is breast cancer, bone cancer, head and neck squamous cell carcinoma, laryngeal and vulvar carcinoma, non-small cell carcinoma, renal cell carcinoma, or prostate cancer. In certain embodiments, the individual is at risk for a hyperproliferative disease, including, cancer, e.g., carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases. This includes individuals having one or more risk factors for developing a hyperproliferative disease, including, growing older; tobacco use; exposure to sunlight and ionizing radiation; contact with certain chemicals; infection with some viruses and bacteria; certain hormone therapies; genetic predisposition; alcohol use; and certain lifestyle choices including poor diet, lack of physical activity, and/or being overweight. In certain embodiments, the individual has been identified as in need of treatment for a hyperproliferative disease. In certain embodiments the invention provides methods for prophylactically reducing MMP-13 expression in

an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to a MMP-13 nucleic acid.

In certain embodiments, the invention provides methods, compounds, and compositions for treating individuals having a type of cancer associated with the upregulation of certain genes at the tumor bone interface. In certain embodiments, the upregulated genes are matrix metallo proteinases (MMP). In certain embodiments, the genes are IBSP; RANKL; MMP-13; BSP; RIKEN; procollagen, type XI, alpha I; Wif1, IGFBP5; hyaluronan receptor RHAMMV5; mMCP1; lumican; complex associated-testis-expressed 1-like; ASF; LOX; cadherin 11; expressed sequence AI844545; expressed sequence AI465480; Cul3; ubiquitin specific protease 9, X chromosome; serinethreonine kinase 3; fatty acid-coenzyme A ligase, long chain 4; and ubiquitin-activating enzyme E1C. In certain embodiments, the gene is MMP-13. In certain embodiments, the type of cancer associated with the upregulation of certain genes, such as MMP-13, at the tumor bone interface is renal cell carcinoma, breast cancer, non small cell lung carcinoma, and prostate cancer.

In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing metastasis of a cancer associated with the upregulation of certain genes, such as MMP-13, at the tumor bone interface to bone. In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing cancer from metastasizing to bone. In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing renal cell carcinoma, breast cancer, non small cell lung carcinoma, and prostate cancer from metastasizing to bone.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to a MMP-13 nucleic acid is accompanied by monitoring of MMP-13 levels in the serum of an individual to determine an individual's response to administration of the antisense compound. An individual's response to administration of the antisense compound is used by a physician to determine the amount and duration of therapeutic intervention.

In certain embodiments, administration of an antisense compound targeted to a MMP-13 nucleic acid results in reduction of MMP-13 expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to a MMP-13 nucleic acid results in reduced cellular growth; reduced tumor growth; reduced tumor volume; reduced osteoclastogenesis; reduced osteolysis, including tumor induced osteolysis; amelioration of

symptoms associated with cancer; and reduction of cancer markers. In certain embodiments, administration of a MMP-13 antisense compound decreases cellular growth, tumor growth, tumor volume, osteolysis, and osteoclastogenesis by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to MMP-13 are used for the preparation of a medicament for treating a patient suffering or susceptible to a hyperproliferative disease.

Certain Combination Therapies

In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease, disorder, or condition as the one or more pharmaceutical compositions of the present invention. In certain embodiments, such one or more other pharmaceutical agents are designed to treat a different disease, disorder, or condition as the one or more pharmaceutical compositions of the present invention. In certain embodiments, such one or more other pharmaceutical agents are designed to treat an undesired side effect of one or more pharmaceutical compositions of the present invention. In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with another pharmaceutical agent to treat an undesired effect of that other pharmaceutical agent. In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with another pharmaceutical agent to produce a combinational effect. In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with another pharmaceutical agent to produce a synergistic effect.

In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared separately. In certain embodiments, one or more other pharmaceutical agents include all-trans retinoic acid, azacitidine, azathioprine, bleomycin, carboplatin, capecitabine, cisplatin,

chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabine, hydroxyurea, idarubicin, imatinib, mechlorethamine, mercaptopurine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, valrubicin, vinblastine, vincristine, vindesine, or vinorelbine.

In certain embodiments, one more pharmaceutical compositions of the present invention are administered with radiation therapy. In certain embodiments, one or more pharmaceutical compositions are administered at the same time as radiation therapy. In certain embodiments, one or more pharmaceutical compositions are administered before radiation therapy. In certain embodiments, one or more pharmaceutical compositions are administered after radiation therapy. In certain embodiments, one or more pharmaceutical compositions are administered at various time points throughout a radiation therapy regimen.

In certain embodiments, radiation therapy is useful for inhibiting osteolysis. In certain embodiments, radiation therapy is useful for increasing overall survival. In certain embodiments, radiation therapy used in conjunction with administration of one or more pharmaceuticals of the present invention is advantageous over using either therapy alone because both radiation therapy and administration with one or more pharmaceuticals can be limited to achieve effective antiproliferative response with limited toxicity.

In certain embodiments, a physician designs a therapy regimen including both radiation therapy and administration of one more pharmaceutical compositions of the present invention. In certain embodiments, a physician designs a therapy regimen including radiation therapy, administration of one or more pharmaceutical compositions of the present invention, and administration of one or more other chemotherapeutic.

EXAMPLES

Non-limiting disclosure and incorporation by reference

While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references recited in the present application is incorporated herein by reference in its entirety.

Example 1: Gene expression profile at the tumor bone interface

Gene expression patterns at the tumor-bone (TB) interface and, separately, the tumor tissue were compared using cDNA microarray. Mammary tumor cells with different metastatic potentials were included in this assay. The results were confirmed by quantitative RT-PCR analysis.

Treatment

Three different mammary adenocarcinoma cell lines (5×10^4 cells each) including, 4T1 having a high metastatic potential, C166 having a moderate metastatic potential, and C166M2 having a low metastatic potential, were mixed in growth-factor reduced Matrigel (BD Biosciences, San Jose, CA) and transplanted onto the calvaria of female BALB/c mice. The cells were directly injected onto the calvaria to mimic the close association of tumor cells and bone.

Four weeks post implantation of tumor cells, mice were euthanized, and tumor tissue and TB-interface tissue samples were collected. The tissues were sectioned and frozen for further analysis.

Microarray analysis

Calcified frozen tissue sections of the TB-interface and the tumor tissue were serially sectioned into 10 μ m thick slices and mounted on slides. At least ten such slides per mouse were micro-dissected, as described in previous publications (Wilson et al, Cancer Res. 2008, 68: 5803-11; Futakuchi *et al*, Cancer Sci. 2009, 100: 71-81). Total RNA was extracted from each micro-dissected population. The RNA samples were pooled and equal amounts of RNA per tissue were amplified using a probe amplification kit (Affymetrix, Santa Clara, CA).

An Affymetrix Mouse Expression Array 430 was used for comparing gene expression profiles between the TB-interface and the tumor tissue. A complete detection and analysis of signals for each chip was performed using Affymetrix GeneChip® Operating Software to generate raw expression data. A signal log ratio algorithm was used to estimate the magnitude of change of a transcript in the TB-interface versus the tumor tissue. The algorithm was calculated by comparing each probe pair on the tumor-bone interface array to the corresponding probe pair on the tumor tissue array, and calculating the mean of the log ratios of probe pair intensities across the two arrays. The tumor tissue probe pair intensity was considered the baseline. For each of the samples from 4T1-injected, C166-injected or C166M2-injected mice, the signal log ratio of the TB-interface versus the tumor tissue was calculated, and the genes were ordered from highest to lowest expression levels.

According to the microarray analysis, 414 genes were upregulated and 27 genes were downregulated at the TB-interface compared to the tumor tissue for all three cell lines. As shown in Table 1, the MMP-13 gene was upregulated at the TB-interface as compared to tumor tissue.

Table 1

Microarray analysis (probe pair signal intensity log₂ ratio) of TB-interface and tumor tissue in tumor-bearing mice

4T1	CI66	CI66M2	Genes
3.90	10.90	6.40	IBSP
4.20	4.60	6.20	RANKL
2.10	4.30	5.50	MMP13
4.10	5.20	2.60	BSP
3.70	0.60	1.10	Mouse RIKEN
2.00	3.30	1.10	Procollagen, type XI, alpha 1
2.20	2.30	3.50	Wif1
1.50	2.40	2.90	IGFBP5
3.40	1.00	1.20	Hyaluronan receptor RHAMMV5
0.80	2.20	3.60	mMCP1
2.40	2.00	2.50	Lumican
2.40	0.90	1.80	Complex associated-testis-expressed 1-like
2.20	2.30	3.50	ASF
1.00	1.10	2.40	LOX
1.90	1.20	0.60	Cadherin 11
2.40	0.80	0.80	Expressed sequence AI844545
2.20	0.70	0.60	Expressed sequence AI465480
2.10	0.60	0.70	Cul3
2.10	0.80	0.70	Ubiquitin specific protease 9, X chromosome
2.00	0.70	0.60	Serine/threonine kinase 3
1.90	0.70	0.50	Fatty acid-Coenzyme A ligase, long chain 4
1.80	0.70	0.80	Ubiquitin-activating enzyme E1C
-0.80	-0.90	-3.90	Histocompatibility 2, class II antigen E beta (H2-Eb1)
-0.80	-0.60	-2.90	Coronin, actin binding protein 1A
-1.00	-0.60	-2.70	Retinoic acid-inducible E3 protein
-1.20	-0.70	-2.70	Y168 human hypothetical protein KIAA0168
-0.80	-0.50	-0.70	Signal transducer and activator of transcription 3
-0.80	-0.30	-0.60	Selectin, endothelial cell, ligand
-0.80	-0.40	-0.60	Forkhead box J2
-0.80	-0.40	-0.60	prostate tumor over expressed gene 1 (Ptov1)
-0.80	-0.30	-0.50	Angio-associated migratory protein, related sequence
-0.90	-0.40	-0.50	Dynein, cytoplasmic, heavy chain 1
-0.90	-0.40	-0.30	LIM domain binding protein 1a

-0.80	-0.40	-1.00	Kruppel-like factor 2 (lung)
-0.80	-0.50	-0.90	RNA polymerase II largest subunit (RPB1) mRNA
-1.00	-0.70	-0.60	Abca7
-1.20	-0.40	-0.80	Adam15
-1.20	-0.50	-0.60	polymeric immunoglobulin receptor 3 precursor
-1.30	-0.70	-1.10	Complement component 4 (within H-2S)
-2.00	-1.40	-1.90	Homeobox D9

Quantitative RT-PCR analysis

RNA extraction was performed by homogenization of tissue samples in liquid nitrogen. Total RNA was extracted using TRIzol® reagent (Invitrogen, CA) following the manufacturer's instructions. RNA concentration was quantified using an ND-1000 Spectrophotometer (Nano Drop Technologies, Wilmington, DE). Five µg of RNA from each sample was used to synthesize the first strand of cDNA. Two µl of 1:100 diluted first strand cDNA was amplified in a 20 µl reaction with SYBR green master mix (Roche, Indianapolis IN) and 10 mM primer mix using a Bio-Rad iCycler (Bio-Rad, Hercules, CA).

The reaction conditions used were as follows: initial denaturation at 95°C for 3 min, followed by amplification cycles with denaturation at 95°C for 60 sec, annealing at 60°C for 60 sec, and extension at 72°C for 60 sec, and finally a long extension at 72°C for 2 min. The primers for MMP-13 were TCCCTGCCCCTTCCCTATGG (forward primer, designated herein as SEQ ID NO: 5) and CTCGGAGCCTGTCAACTGTGG (reverse primer, designated herein as SEQ ID NO: 6). The fluorescence intensity of double-strand specific SYBR Green, reflecting the amount of formed PCR-product, was monitored at the end of each elongation step. The results were normalized with GAPDH expression for relative gene expression analysis.

MMP-13 mRNA expression at the TB-interface was observed to be 2718 % higher than that of the tumor tissue. This finding was observed with all the adenocarcinoma cell lines and is an average of three independent experiments conducted with each cell line. These results confirm upregulation of MMP-13 mRNA expression at the TB-interface compared to tumor tissue in tumor-bearing mice.

Example 2: Analysis of MMP-13 mRNA expression kinetics and its association with tumor-induced osteolysis

The kinetics of MMP-13 expression and its association with tumor-induced osteolysis and osteoclast activation was examined.

Treatment

C166 adenocarcinoma cells (5×10^4), with moderate metastatic potential, were mixed in growth-factor reduced Matrigel and injected onto the calvaria of female BALB/c mice. The mice were euthanized at week 2, week 3, or week 4 after tumor transplantation and examined for bone destruction, osteoclast numbers, and MMP-13 mRNA expression at the TB-interface compared to tumor tissue.

RNA analysis

RNA extraction and analysis was performed, as described in Example 1. As shown in Table 2, MMP-13 mRNA expression levels at the TB-interface increased over time, and there was a higher level of MMP-13 mRNA expression at the TB-interface compared to the tumor tissue. The results are expressed as percentage change in MMP-13 mRNA levels and were normalized to GAPDH expression for relative gene expression analysis.

Table 2
MMP-13 expression levels (%) at the TB-interface and tumor tissue over time

	Week 2	Week 3	Week 4
Tumor tissue	6	3	1
TB-interface	54	91	72

Bone destruction quantification and quantification of TB-interface osteoclast numbers

The severity of bone destruction was quantified by measuring the bone destruction index (BDI), which is the ratio of the length of the bone that is destroyed by the tumor to the total length of the bone at the TB-interface. As shown in Table 3, there was increased bone destruction due to tumor-induced osteolysis over time.

Quantification of osteoclast numbers at the TB-interface was achieved by staining tissue sections with tartrate-resistant acid phosphatase (TRAP) (Sigma Chemicals, St. Louis, MO), which specifically stains osteoclast cells (Filgueira, *J Histochem Cytochem* 52:411–414, 2004). Briefly, de-paraffinized slides were rinsed with deionized water and then incubated with TRAP-containing buffer at 37°C for one hour. Next, the slides were then rinsed with deionized water and counter-

stained with Gill3 Hematoxylin solution for two minutes. The stained samples were mounted with water. Immunostained sections were examined under a Nikon light microscope and the number of TRAP-positive multinucleated cells at the TB-interface was enumerated at a magnification of 400X for each lesion. The total number of osteoclasts was then divided by the length of the tumor-bone interface to get the number of osteoclasts per mm of TB-interface. As shown in Table 3, there was an increase in osteoclast numbers over time at the TB-interface.

Table 3
BDI and osteoclast number (per mm) at the TB-interface

	Week 2	Week 3	Week 4
BDI	28	46	60
Osteoclast number	44	52	65

Example 3: Antisense inhibition of rodent matrix metalloproteinase 13 (MMP-13) in TM-3 cells

Antisense oligonucleotides targeted to a MMP-13 nucleic acid were tested for their effects on MMP-13 mRNA *in vitro*. Cultured TM-3 cells at a density of 5,000 cells per well were transfected using lipofectin reagent with 150 nM antisense oligonucleotide for 4 hours. After a recovery period of approximately 24 hours, RNA was isolated from the cells and MMP-13 mRNA levels were measured by quantitative real-time PCR. MMP-13 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of MMP-13, relative to untreated control cells.

The chimeric antisense oligonucleotides in Tables 4 and 5 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on the 5' and 3' sides by wings comprising 5 nucleotides each. Each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines.

Each gapmer listed in Table 4 is targeted to murine MMP-13 mRNA (GENBANK Accession No. NM_008607.1, designated herein as SEQ ID NO: 7). "Murine Target start site" indicates the 5'-

most nucleotide to which the gapmer is targeted. “Murine Target stop site” indicates the 3’-most nucleotide to which the gapmer is targeted.

Each gapmer listed in Table 5 is targeted to rat MMP-13 mRNA (GENBANK Accession No. M60616.1, designated herein as SEQ ID NO: 1) or rat MMP-13 gene sequence (GENBANK Accession No. BE127506.1, designated herein as SEQ ID NO: 2) or rat MMP-13 gene sequence (GENBANK Accession No. AW914210.1, designated herein as SEQ ID NO: 3). “Rat Target start site” indicates the 5’-most nucleotide to which the gapmer is targeted. “Rat Target stop site” indicates the 3’-most nucleotide to which the gapmer is targeted.

Certain antisense oligonucleotides of Tables 4 and 5 are cross reactive with the human MMP-13 mRNA (GENBANK Accession NM_002427.2, incorporated herein as SEQ ID NO: 4). The number of mismatched nucleobases between the rodent oligonucleotide and the human MMP-13 mRNA sequence are indicated. “Human Target Start Site” indicates the 5’-most nucleotide in the human mRNA to which the antisense oligonucleotide is targeted. “Human Target Stop Site” indicates the 3’-most nucleotide in the human mRNA to which the antisense oligonucleotide is targeted. ‘Mismatches’ indicates the number of nucleobases by which the rodent oligonucleotide is mismatched with the human gene sequence. The designation “n/a” indicates that there was greater than 3 mismatches between the rodent oligonucleotide and the human gene sequence.

Table 4

Inhibition of MMP-13 mRNA levels by chimeric murine antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 7

ISIS No.	Murine Target Start Site	Murine Target Stop Site	Sequence	% inhibition	SEQ ID NO	Human target start site	Human target stop site	Mis-matches
262348	28	47	TCAACAAGAA GAAGGTGGCC	91	8	n/a	n/a	n/a
262349	73	92	CATCATCACCA TAGGGAAGG	72	9	91	110	3
262350	84	103	GTCATCATCAT CATCATCAC	92	10	n/a	n/a	n/a
262351	124	143	TCAAGTAGTGC TCTGCAAAC	84	11	139	158	2
262352	129	148	TGATTTCAAGT AGTGCTCTG	91	12	144	163	3

262353	163	182	TCAGGATTCCC GCAAGAGTC	97	13	178	197	3
262354	178	197	TCACTGTAGAC TTCTTCAGG	86	14	n/a	n/a	n/a
262356	248	267	TCATCAAGTTT GCCAGTCAC	91	15	263	282	1
262357	272	291	TTTCTCATGAT GTCTAAGGT	72	16	287	306	3
262358	292	311	CAGGCACTCCA CATCTTGGT	87	17	n/a	n/a	n/a
262360	339	358	TTGGGACCATT TGAGTG TTC	97	18	354	373	3
262361	401	420	TTCTCCACTTC AGAATGGGA	94	19	n/a	n/a	n/a
262362	411	430	TCTGAAGGCCT TCTCCACTT	94	20	n/a	n/a	n/a
262363	421	440	TGAAGGCTTTT CTGAAGGCC	80	21	436	455	2
262364	431	450	GACCAGACCTT GAAGGCTTT	65	22	446	465	2
262365	441	460	TGTCACATCAG ACCAGACCT	84	23	n/a	n/a	n/a
262366	518	537	AAGTCACCATG TTCTTTAGT	77	24	n/a	n/a	n/a
262367	528	547	AAATGGGTAG AAGTCACCAT	85	25	543	562	1
262368	533	552	CCATCAAATGG GTAGAAGTC	68	26	548	567	0
262369	554	573	TGTGCCAGAA GACCAGAAGG	70	27	n/a	n/a	n/a
262370	564	583	AGGAAAAGCG TGTGCCAGAA	46	28	579	598	3
262371	574	593	TTGGTCCAGGA GGAAAAGCG	53	29	589	608	2
262372	612	631	GGTTTCATCAT CATCAAAAT	17	30	627	646	0

262373	619	638	TTGTCCAGGTT TCATCATCA	85	31	634	653	0
262374	662	681	TCATGGGCAGC AACAATAAA	88	32	677	696	2
262376	727	746	TATAGATGGG AAACATCAGG	54	33	n/a	n/a	n/a
262377	737	756	CCAGTGTAGGT ATAGATGGG	55	34	752	771	3
262378	747	766	ATGGCTTTTGC CAGTGTAGG	85	35	762	781	2
262383	845	864	CACTTCTCTGG TGTTTTGGG	77	36	n/a	n/a	n/a
262384	855	874	GGCTGGGTCAC ACTTCTCTG	81	37	n/a	n/a	n/a
262385	874	893	TAATGGCATCA AGGGATAGG	87	38	889	908	1
262386	914	933	AATCTGTCTTT AAAGATCAT	44	39	929	948	0
262387	919	938	AGAAGAATCT GTCTTTAAAG	57	40	934	953	0
262388	944	963	TCAACCTGCTG AGGGTGCAG	85	41	959	978	1
262389	983	1002	AGTTCTGGCCA AAAGGACTT	37	42	998	1017	2
262390	989	1008	TTGGGAAGTTC TGGCCAAAA	79	43	1004	1023	0
262391	998	1017	TCCACATGGTT GGGAAGTTC	99	44	1013	1032	3
262392	1055	1074	AATTTTCTCCC TCTAAAGAT	55	45	1070	1089	2
262393	1106	1125	GATATTTTCT GGGATAACC	58	46	1121	1140	1
262394	1116	1135	TCCCAGGTCAG ATATTTTTC	65	47	1131	1150	3
262395	1182	1201	GAAGAGGGTC TTCCCCGTGT	92	48	n/a	n/a	n/a

262396	1210	1229	CATAACTCCAC ACGTGGTTC	91	49	n/a	n/a	n/a
262397	1237	1256	CTTTGTCCATA GTCTGGTTA	97	50	1252	1271	3
262398	1302	1321	CTCATAGACAG CATCTACTT	51	51	1317	1336	0
262399	1307	1326	TTTTTCTCATA GACAGCATC	81	52	1322	1341	0
262400	1314	1333	ATAGCCATTTT TCTCATAGA	92	53	1329	1348	1
262401	1324	1343	AAAAGTAGAT ATAGCCATTT	70	54	1339	1358	2
262402	1337	1356	ATGGGCCCATT GAAAAAGTA	63	55	1352	1371	3
262403	1347	1366	TTCAAACGTGA TGGGCCCAT	99	56	1362	1381	2
262404	1352	1371	CTGTATTCAAA CTGTATGGG	66	57	1367	1386	0
262405	1362	1381	ACTCCAGATAC TGTATTCAA	95	58	1377	1396	1
262406	1372	1391	CAATGCGATTA CTCCAGATA	93	59	1387	1406	3
262407	1382	1401	ATGACTCTCAC AATGCGATT	81	60	n/a	n/a	n/a
262409	1504	1523	GCTCTCCCCTG ATATCTCCA	93	61	n/a	n/a	n/a
262410	1509	1528	ACTAAGCTCTC CCCTGATAT	47	62	n/a	n/a	n/a
262411	1514	1533	ACAGAACTAA GCTCTCCCCT	81	63	1532	1551	3
262413	1576	1595	TTCCAGCCACG CATAGTCAT	94	64	1594	1613	0
262414	1581	1600	TGTGGTTCCAG CCACGCATA	95	65	1599	1618	0
262416	1633	1652	AGAATCAGGT GATCCTTGGG	78	66	n/a	n/a	n/a

262417	1674	1693	TGCTGTGGGTT ATTATCAAT	99	67	n/a	n/a	n/a
262418	1777	1796	CAATTCAGTTA TATAAATAT	9	68	n/a	n/a	n/a
262419	1783	1802	TAAAGACAATT CAGTTATAT	52	69	n/a	n/a	n/a
262420	1788	1807	TTTTGTAAAGA CAATTCAGT	67	70	n/a	n/a	n/a
262421	2496	2515	CATCAGTAAGC ACCAAGTGT	78	71	n/a	n/a	n/a
262422	2501	2520	TCACACATCAG TAAGCACCA	98	72	n/a	n/a	n/a
262423	2606	2625	TTTTTATTAGA AACAACATA	53	73	2681	2700	1

Table 5

Inhibition of MMP-13 mRNA levels by chimeric rat antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 or 2

ISIS No.	Rat Target Start Site	Rat Target Stop Site	Rat Target Sequence	Sequence	% inhibition	SEQ ID NO	Human target start site	Human target stop site	Mis-matches
262355	188	207	1	AAAGACTGCAT TTCTCGGAG	70	74	230	249	0
262359	296	315	1	GTTTCGAGGGAA AACATTGTA	76	75	338	357	0
262375	677	696	1	CCAGGGTCCTTG GAGTGGTC	93	76	719	738	0
262379	770	789	1	GGACCATAGAG AGACTGGAT	7	77	812	831	0
262380	775	794	1	CTCCTGGACCAT AGAGAGAC	63	78	817	836	0
262381	780	799	1	TTCATCTCCTGG ACCATAGA	53	79	822	841	0
262382	785	804	1	GGGTCTTCATCT CCTGGACC	64	80	827	846	0

262408	1392	1411	1	GAAAGATGTTT AACACCACA	62	81	n/a	n/a	n/a
262412	1496	1515	1	CTGAAGCTTGTT CACAGAAC	81	82	1545	1564	0
262415	1556	1575	1	TTCAATGTGGTT CCAGCCAC	76	83	1604	1623	0
262424	9	28	2	CAGAATAGCTG AATGCATGG	52	84	n/a	n/a	n/a
262425	32	51	2	GGATCCACTAG TTCTAGAGC	8	85	n/a	n/a	n/a

Example 4: Effect of antisense inhibition of matrix metalloproteinase 13 (MMP-13) in tumor-bearing mice

The effect of inhibition of MMP-13 mRNA expression with antisense oligonucleotides was examined in C166 tumor-bearing mice. mRNA and protein expression of osteolysis-related genes, as well as their activity, were assessed. The BDI and osteoclast numbers at the TB-interface, as well as tumor growth patterns were also evaluated. This experiment was repeated twice.

Treatment

C166 adenocarcinoma cells (5×10^4), with moderate metastatic potential, were mixed in growth-factor reduced Matrigel and transplanted onto the calvaria of female BALB/c mice. Seven days after tumor transplantation, the mice were randomly divided into two treatment groups. The first treatment group was injected with MMP-13 antisense oligonucleotide, ISIS 262403 (SEQ ID NO: 56). The second treatment group was injected with control oligonucleotide, ISIS 347526 (5'-TCTTATGTTTCCGAACCGTT-3', a 5-10-5 MOE gapmer, designated herein as SEQ ID NO: 86), having no known homology to any mouse gene. The oligonucleotides were dissolved in PBS and were administered to the two treatment groups by intraperitoneal injection at a dose of 50 mg/kg/day for 5 days. Treatment was withheld for two days followed by administration for another 4 days. Tumor growth was monitored and mice were sacrificed on day 28. Tumor tissue and TB-interface samples were collected and processed for further analysis. The data presented is the average of three independent experiments with similar results.

RNA analysis

RNA extraction and analyses was performed as described in Example 1. MMP-13 mRNA expression was normalized to GAPDH mRNA expression.

MMP-13 mRNA expression was assessed at the TB-interface. As shown in Table 6, MMP-13 mRNA expression in mice treated with ISIS 262403 was inhibited compared to the control oligonucleotide-treated group.

RANKL mRNA expression levels were also assessed at the TB-interface with forward primer TTAGCATTCAGGTGTCCAACC (designated herein as SEQ ID NO: 87) and reverse primer CGTGGGCCATGTCTCTTAGTA (designated herein as SEQ ID NO: 88). As shown in Table 6, treatment of tumor-bearing mice with antisense oligonucleotides targeting MMP-13 resulted in significant decrease in RANKL mRNA expression at the TB-interface compared to the control oligonucleotide-treated group.

MMP-9 mRNA expression levels were also assessed at the TB-interface with forward primer CATTCGCGTGGATAAGGAGT (designated herein as SEQ ID NO: 89) and reverse primer TCACACGCCAGAAGAATTTG (designated herein as SEQ ID NO: 90). As shown in Table 6, treatment of tumor-bearing mice with antisense oligonucleotides targeting MMP-13 resulted in significant decrease in MMP-9 mRNA expression at the TB-interface compared to the control oligonucleotide-treated group.

TGF-beta mRNA expression levels were also assessed at the TB-interface with forward primer CGCCATCTATGAGAAAACCAA (Designated herein as SEQ ID NO: 91) and reverse primer GACGTCAAAGACAGCCACTC (designated herein as SEQ ID NO: 92). As shown in Table 6, treatment of tumor-bearing mice with antisense oligonucleotides targeting MMP-13 resulted in significant decrease in TGF-beta mRNA expression at the TB-interface compared to the control oligonucleotide-treated group.

The mRNA expression levels at the TB interface are expressed as percent increase or decrease of expression levels compared to that in the tumor tissue (normalized to 100%).

Table 6
mRNA expression levels (%) at TB-interface compared to tumor tissue

	MMP-13	RANKL	MMP-9	TGF-beta
ISIS 347526	+16414	+1024	+209	+136
ISIS 262403	+73	-26	-68	-55

Protein analysis

RANKL and osteoprotegerin (OPG) protein expression levels were also measured at the TB-interface. Quantitative determination of RANKL and OPG levels at the TB-interface and tumor tissue was performed using a commercially available ELISA kit (Biomedica, Vienna, Austria), according to the manufacturer's instructions. Briefly, pre-coated wells were incubated with samples or recombinant standard and biotinylated antibody for 3 hours. After washing, the reactivity was detected using streptavidin-HRP conjugate and tetramethylbenzidine substrate solution. The reaction was stopped and absorbance was determined at 450 nm, with correction at 540 nm using an ELx800 ELISA plate reader (BioTek, Winooski, VT). The sensitivity of these kits was 0.4 pmol/l for RANKL and 0.14 pmol/l for OPG.

The results are presented in Table 7 as a percentage increase over that of the tumor tissue. As shown in Table 7, there was a decrease in RANKL protein levels in the ISIS 262403 treated mice compared to the control. This result corresponds with the decrease in mRNA expression levels. Though OPG protein expression levels were similar in the treatment and control groups, the RANKL: OPG ratio was significantly decreased in tumor-bearing mice treated with ISIS 262403. The RANKL:OPG ratio is a measurement of osteoclastogenesis and osteolysis. Therefore, treatment of tumor-bearing mice with antisense oligonucleotides targeting MMP-13 shows decreased tumor-induced osteoclastogenesis and osteolysis compared to the control group. The protein levels are expressed in absorbance units representing optical density, and were normalized to a blank PBS control.

Table 7
RANKL and OPG protein expression levels at the TB-interface of the treatment groups

	RANKL	OPG	RANKL: OPG ratio
ISIS 347526	183	241	0.76
ISIS 262403	134	228	0.59

Measurement of tumor growth

Tumor volume measurements were taken weekly using Vernier Callipers. As shown in Table 8, tumor growth was inhibited in tumor-bearing mice treated with antisense oligonucleotides targeting MMP-13 compared to that of the control oligonucleotide group.

Table 8
Weekly tumor volume measurements (mm³) of the treatment groups

	Week 1	Week 2	Week 3	Week 4
ISIS 347526	271	541	605	728
ISIS 262403	109	202	252	352

Quantification of bone destruction and osteoclast numbers

The severity of bone destruction was quantified by measuring the bone destruction index (BDI), which is the ratio of the length of the bone that is destroyed by the tumor to the total length of the bone at the TB-interface. As shown in Table 9, bone destruction was significantly decreased in mice treated with ISIS 262403 compared to the control oligonucleotide group.

Osteoclast numbers were quantified using TRAP staining, as described in Example 2. As shown in Table 9, osteoclast numbers were significantly decreased in mice treated with ISIS 262403 compared to the control oligonucleotide group. Osteoclasts are bone-resorbing cells which are derived from macrophage or myeloid lineage progenitors (Teitelbaum S.L. *Science* 2000. 289: 1504–1508). Osteoclasts accumulate at sites of bone inflammation, causing osteoporosis. Accordingly, there is a strong correlation between the number of osteoclasts at the TB-interface and bone destruction. Therefore, the reduced number of osteoclasts indicates that antisense inhibition of MMP-13 is useful in reducing bone destruction.

Table 9
BDI and osteoclast number (per mm) in the treatment groups

	BDI	Osteoclast number
ISIS 347526	63	82
ISIS 262403	0	13

Evaluation of MMP-9 gelatinolytic activity

Measurement of active MMP-9 versus pro-MMP-9 was determined by gelatin zymography. Protein was extracted using T-PER tissue protein extractor solution (Pierce, Rockford, IL) following the manufacturer's provided protocol. Protein samples were quantified using a BCA protein assay kit (Pierce). Fifty µg total protein isolated from either the TB-interface or tumor tissue was subjected to electrophoresis on a 10% (w/v) polyacrylamide SDS gel containing 1 mg/mL porcine gelatin (Sigma-Aldrich, St. Louis, MO). At the completion of electrophoresis, the gel was washed with 2.5% Triton X-100 buffer for 30 minutes. After rinsing, the gel was incubated for 12 hours at 37°C in incubation buffer containing 50 mM tris-HCl (pH 7.4), 150 mM NaCl, 10 mM CaCl₂, and 0.05% (w/v) NaN₃. The gel was then stained using 0.025% Coomassie brilliant blue (Bio-Rad, Hercules, CA) and photographed using a Multi Image Light Cabinet (Alpha Innotech Corporation, San Leandro, CA). The volume of gelatinolytic activity at 92 kDa, representing the active MMP-9 band, was evaluated using ImageQuant® 5.1 (Molecular Dynamics, Sunnyvale, CA).

MMP-9 to pro-MMP-9 ratio was assessed by gelatinolytic activity. As shown in Table 10, there was a significant decrease in gelatinolytic activity in samples from mice treated with antisense oligonucleotides targeting MMP-13 compared to that in the control oligonucleotide group. The results are based on densitometric scanning analysis of the active and pro-MMP-9 bands compared to an area on the gel with no band (or background).

Previous studies have demonstrated that pro- and active MMP-9 is upregulated during tumor-bone interaction leading to osteoclast recruitment at the TB-interface which, consequently, results in bone damage (Wilson T.J. et al., Cancer Res. 2008. 68: 5803-11). As shown in Table 10, antisense inhibition of MMP-13 causes a decrease in MMP-9 activity. Therefore, the reduction of MMP-9 activity by antisense inhibition of MMP-13 may be beneficial in reducing osteoclast recruitment, thus, preventing bone destruction.

Table 10
Active MMP-9: pro-MMP9 ratio in the treatment groups

	Active: pro-MMP-9
ISIS 347526	116
ISIS 262403	37

Evaluation of TGF-beta signaling

Measurement of TGF-beta expression and activity was determined by immunohistochemical analysis of phosphorylated Smad2 (pSmad2). Sections were blocked using goat serum diluted 1:500 for one hour at room temperature. Sections were then incubated overnight at 4°C with antibody directed against pSmad2 (Ser 465/467, Cell Signaling Technology, Danvers, MA) diluted 1:50 in blocking solution. After washing, sections were incubated for one hour at room temperature with biotinylated anti-rabbit IgG diluted 1:500. As shown in Table 11, there was a significant decrease in pSmad2 staining index and, therefore, of TGF-beta signaling in samples from mice treated with antisense oligonucleotides targeting MMP-13 compared to that in mice treated with the control oligonucleotide. The results are expressed as the number of pSmad2 positive cells at TB interface normalized to that with a control antibody.

TGF-beta signaling plays a role in tumor-bone interaction by promoting tumor growth and osteoclast activation (Futakuchi M. et al., *Cancer Sci.* 2009. 100: 71-81). Since antisense inhibition of MMP-13 causes a significant decrease in pSmad2 activity, this suggests that MMP-13 levels at the TB-interface potentiate TGF-beta signaling, which ultimately contributes to osteolytic bone metastasis. Therefore, antisense inhibition of MMP13 is beneficial in preventing bone resorption and tumor metastasis.

Table 11
pSmad2 staining index as a measure of TGF-beta signaling in the treatment groups

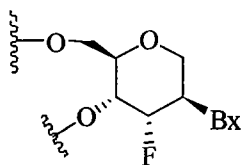
	pSmad2 positive index
ISIS 347526	147
ISIS 262403	18

CLAIMS

What is claimed is:

1. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NO: 8 to 85.
2. The compound of claim 1, consisting of a single-stranded modified oligonucleotide.
3. The compound of claim 2, wherein the nucleobase sequence of the modified oligonucleotide is 100% complementary to a nucleobase sequence of SEQ ID NO: 4.
4. The compound of claim 2, wherein at least one internucleoside linkage is a modified internucleoside linkage.
5. The compound of claim 4, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.
6. The compound of claim 1, wherein at least one nucleoside comprises a modified sugar.
7. The compound of claim 6, wherein at least one modified sugar is a bicyclic sugar.
8. The compound of claim 7, wherein each of the at least one bicyclic sugar comprises a 4'-(CH₂)_n-O-2' bridge, wherein n is 1 or 2.
9. The compound of claim 7, wherein each of the at least one bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.

10. The compound of claim 6, wherein at least one modified sugar comprises a 2'-O-methoxyethyl group.
11. The compound of claim 1, comprising at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.
12. The compound of claim 11, wherein each of the at least one tetrahydropyran modified nucleoside has the structure:



wherein Bx is an optionally protected heterocyclic base moiety.

13. The compound of claim 2, wherein at least one nucleoside comprises a modified nucleobase.
14. The compound of claim 13, wherein the modified nucleobase is a 5-methylcytosine.
15. The compound of claim 1, wherein the modified oligonucleotide comprises:
 a gap segment consisting of linked deoxynucleosides;
 a 5' wing segment consisting of linked nucleosides;
 a 3' wing segment consisting of linked nucleosides;
 wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.
16. The compound of claim 15, wherein the modified oligonucleotide comprises:
 a gap segment consisting of ten linked deoxynucleosides;
 a 5' wing segment consisting of five linked nucleosides;
 a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned immediately adjacent and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

17. The compound of claim 16, wherein each cytosine is a 5-methylcytosine.
18. The compound of claim 2 or 17, wherein the modified oligonucleotide consists of 20 linked nucleosides.
19. A composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 8 to 85 or a salt thereof and a pharmaceutically acceptable carrier or diluent.
20. A method of reducing osteoclastogenesis in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.
21. A method of reducing osteolysis in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.
22. A method of inhibiting tumor growth in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.
23. A method of decreasing bone destruction in an animal in need thereof, comprising:

administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

24. A method of reducing osteoclasts in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

25. A method of decreasing gelatinolytic activity in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

26. A method of reducing TGF-beta signaling in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

27. A method comprising reducing the risk for osteoclastogenesis in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

28. A method comprising treating cancer in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

29. A method of reducing osteoclastogenesis in an animal in need thereof, comprising:

administering to the animal a therapeutically effective amount of the composition of claim 19.

30. A method of reducing osteolysis in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.

31. A method of inhibiting tumor growth in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.

32. A method of decreasing bone destruction in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.

33. A method of reducing osteoclasts in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.

34. A method of decreasing gelatinolytic activity in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.

35. A method of reducing TGF-beta signaling in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.

36. A method comprising reducing the risk for osteoclastogenesis in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.

37. A method comprising treating cancer in an animal in need thereof, comprising:
administering to the animal a therapeutically effective amount of the composition of claim 19.
38. A method of inhibiting expression of any of the group consisting of MMP13, RANKL, MMP9, and TGF beta in cells or tissues comprising contacting the cells or tissue with the compound of claim 1.
39. The method of claim 38, wherein the cells or tissues are human cells or tissues.
40. The method of claim 39, wherein the cells or tissues are at the tumor bone interface.
41. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 230 to 249 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.
42. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 338 to 357 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.
43. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 548 to 567 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

44. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 627 to 653 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

45. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 719 to 738 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

46. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 812 to 846 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

47. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 929 to 953 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

48. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1004 to 1023 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

49. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1317 to 1341 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

50. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1367 to 1386 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

51. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1545 to 1564 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

52. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1594 to 1623 of SEQ ID NO: 4; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 4.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/30208

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/11; C07H 21/04 (2011.01)

USPC - 514/44A; 536/24.5

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC: 514/44A; 536/24.5

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

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

PubWEST (JPAB, EPAB, USPT, PGPB); Google Scholar; Matrix Metalloproteinase 13 (MMP-13; collagenase 3), human, murine, rat, antisense, siRNA, shRNA, single-stranded oligonucleotide, phosphorothioate, bicyclic sugar, 2'-O-methoxyethyl (2-MOE or 2'-MOE or MOE), tetrahydropyran (THP). GenCore 6.3: SEQ ID NO: 8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 2007/0050146 A1 (BENTWICH et al.) 1 March 2007 (01.03.2007) para [0045], [0069], [0125], pg 49 table 9, SEQ ID NO: 176931	1-2, 4-5, 13, 19 6-12, 14-18
Y	WO 2009/061851 A2 (FREIER et al.) 14 May 2009 (14.05.2009) pg 4 ln 7-9, pg 6 ln 7-19, pg 7 ln 10-11, pg 23 ln 5-10, pg 32 ln 3-19, pg 33 ln 9-12	6-12, 14-18
Y	WO 2009/023855 A2 (ALLERSON et al.) 19 February 2009 (19.02.2009) pg 7 ln 5-6	12

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

* Special categories of cited documents:

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

19 July 2011 (19.07.2011)

Date of mailing of the international search report

28 JUL 2011

Name and mailing address of the ISA/US

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

International application No.

PCT/US 11/30208

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-19 and 41-52, drawn to a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a specified nucleobase sequence. The first invention is restricted to SEQ ID NO:8. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. Due to the number of sequences in this application, an additional invention(s) of Group I+ will be defined as necessary depending on Applicant's ultimate payment of additional fees. The additional sequences will be searched if applicant pays for each additional sequence or shows that the sequences share a special technical feature, i.e. a common structure or feature that defines a contribution over the prior art.

[NOTE: Claims 3 and 41-52 were excluded from the first invention, because they are drawn to a non-elected subject matter.]

-----continued in Extra Sheet-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-2, 4-19, restricted to SEQ ID NO: 8

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/30208

***** Supplemental Box *****

Continuation of Box III (Lack of Unity of Invention)

Note that each additional sequence to be searched must be specified by the Applicant in the response to this invitation and must either (1) have an additional invention fee paid or (2) have a showing that the sequences share a common structure or feature that defines a contribution over the prior art. The exact claims searched will depend on Applicant's election.

Group II, claims 20-40, drawn to a method comprising: administering to the animal a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I+ do not include the inventive concept of method comprising: administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a MMP-13 nucleic acid, as required by Group II.

The inventions of Group II do not include the inventive concept of modified oligonucleotide of a specified SEQ ID NO., as required by Group I+.

The inventions of Group I+ share the technical feature of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of a specified nucleobase sequence. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2009/0099121 A1 to McSWIGGEN et al. (hereinafter "McSwiggen") that teaches a compound comprising a modified oligonucleotide consisting of 19 linked nucleosides (pg 93 SEQ ID NO: 187: guccagguucaucau) and comprising a nucleobase sequence comprising 14 contiguous nucleobases of a nucleobase sequences recited in SEQ ID NO: 30 (McSwiggen's SEQ ID NO: 187, 6-gguucaucau-19 has 100% identity with the claimed SEQ ID NO: 30, 1-GGTTTCATCATCAT-14; para [0034] "In one embodiment, the invention features one or more chemically modified siNA constructs having specificity for MMP13 expressing nucleic acid molecules, such as RNA encoding a MMP13 protein. In one embodiment, the invention features a RNA based siNA molecule (e.g., an siNA comprising 2'-OH nucleotides) having specificity for MMP13 expressing nucleic acid molecules that includes one or more chemical modifications described herein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, (e.g., RNA based siNA constructs), are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds"). As said compound was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Group I+ is the specific nucleic acid sequence recited therein. The inventions do not share a special technical feature, because 1) the claimed SEQ ID NO:4 was known in the art at the time of the invention, as evidenced by WO 2002/78516 A2 to Wang, et al. (hereinafter "Wang") (SEQ ID NO:2906, Matrix metalloproteinase 13 full length cDNA sequence), and 2) no significant structural similarities can readily be ascertained among the amino acid sequences. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.