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(54) **Title**: METHODS FOR MODULATING KALLIKREIN (KLKB1) EXPRESSION

(57) **Abstract**: Disclosed herein are methods for decreasing kallikrein and treating or preventing inflammatory conditions in an individual in need thereof. Examples of disease conditions that can be ameliorated with the administration of antisense compounds targeted to kallikrein include hereditary angioedema (HAE). Methods for inhibiting kallikrein can also be used as a prophylactic treatment to prevent individuals at risk for developing an inflammatory condition, such as, hereditary angioedema.

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METHODS FOR MODULATING KALLIKREIN (KLKB1) 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 BIOL0155WOSEQ.txt created June 6, 2011, which is 217 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

Provided are methods for reducing expression of kallikrein mRNA and protein in an animal. Such methods are useful to treat, prevent, or ameliorate inflammatory conditions, including hereditary angioedema (HAE).

Background

Inflammation

Inflammation is a complex biological process of the body in response to an injury or abnormal stimulation caused by a physical, chemical, or biological stimulus. Inflammation is a protective process by which the body attempts to remove the injury or stimulus and begins to heal affected tissue in the body.

The inflammatory response to injury or stimulus is characterized by clinical signs of increased redness (*rubor*), temperature (*calor*), swelling (*tumor*), pain (*dolor*) and/or loss of function (*functio laesa*) in a tissue. Increased redness and temperature is caused by vasodilation leading to increased blood supply at core body temperature to the inflamed tissue site. Swelling is caused by vascular permeability and accumulation of protein and fluid at the inflamed tissue site. Pain is due to the release of chemicals (e.g. bradykinin) at the inflamed tissue site that stimulate nerve endings. Loss of function may be due to several causes.

Inflammation is now recognized as a type of non-specific immune response to an injury or stimulus. The inflammatory response has a cellular component and an exudative component. In the cellular component, resident macrophages at the site of injury or stimulus initiate the inflammatory response by releasing inflammatory mediators such as TNFalpha, IFNalpha IL-1, IL-6, IL12, IL-18 and others. Leukocytes are then recruited to move into the inflamed tissue area and perform various

functions such as release of additional cellular mediators, phagocytosis, release of enzymatic granules, and other functions. The exudative component involves the passage of plasma fluid containing proteins from blood vessels to the inflamed tissue site. Inflammatory mediators such as bradykinin, nitric oxide, and histamine cause blood vessels to become dilated, slow the blood flow in the vessels, and increase the blood vessel permeability, allowing the movement of fluid and protein into the tissue. Biochemical cascades are activated in order to propagate the inflammatory response (e.g., complement system in response to infection, fibrinolysis and coagulation systems in response to necrosis due to a burn or trauma, kinin system to sustain inflammation) (Robbins Pathologic Basis of Disease, Philadelphia, W.B Saunders Company).

Inflammation can be acute or chronic. Acute inflammation has a fairly rapid onset, quickly becomes severe, and quickly and distinctly clears after a few days to a few weeks. Chronic inflammation can begin rapidly or slowly and tends to persist for weeks, months, or years with a vague and indefinite termination. Chronic inflammation can result when an injury or stimulus, or products resulting from its presence, persists at the site of injury or stimulation and the body's immune response is not sufficient to overcome its effects.

Inflammatory responses, although generally helpful to the body to clear an injury or stimulus, can sometimes cause injury to the body. In some cases, a body's immune response inappropriately triggers an inflammatory response where there is no known injury or stimulus to the body. In these cases, categorized as autoimmune diseases, the body attacks its own tissues causing injury to its own tissues.

Hereditary angioedema

Hereditary angioedema (HAE) is a rare inflammatory disease characterized by recurrent episodes of swelling around the head and extremities (Zuraw, B.L. N. Engl. J. Med. 359: 1027-36, 2008). Angioedema attacks occur with unpredictable frequency and are typically focused on the skin, and gastric, oropharyngeal, and laryngeal mucosas. Asphyxiation due to laryngeal swelling can result in mortality. HAE is caused by deficiency or malfunction of the serine protease inhibitor C1-INH (Kaplan, A.P. et al. J. Allergy Clin. Immunol. 109: 195-209, 2002). C1-INH is the primary inhibitor of coagulation factors 12 and 11 (Factor 11) of the intrinsic coagulation pathway as well as plasma kallikrein (Gigli, I. et al. J. Immunol. 104:574-581, 1970). C1-INH mediated inhibition of plasma kallikrein and Factor 12 results in inactivation of the kallikrein pathway and decreased levels of bradykinin (BK). C1-INH deficiency or dysfunction results in overproduction of BK, which is

the mechanism by which HAE attacks are believed to occur. Type III HAE has been linked with mutations in the Factor 12 gene, which encodes coagulation protein Factor 12 (Cichon, S. et al. Am. J. Hum. Genet. 79: 1098-1104, 2006).

The kinin-kallikrein pathway consists of several proteins that play a role in inflammation, blood pressure control, coagulation, and pain. Plasma prekallikrein is the precursor of plasma kallikrein, which in turn liberates kinins from kininogens and also generates plasmin from plasminogen. Plasma prekallikrein is converted to plasma kallikrein by Factor 12a by the cleavage of an internal Arg-Ile peptide bond. Plasma prekallikrein, in turn, is the product of the KLKB1 gene (MacKenzie, J.A. et al. Appl. Physiol. Nutr. Metab. 35: 518-525, 2010. Plasma kallikrein works in association with Factors 11 and 12.

There is currently no animal model which directly replicates HAE. However, the increased vascular permeability associated with HAE has been replicated in rodent models with agents such as the angiotensin converting enzyme (ACE) inhibitor captopril, as well as the C1-INH knockout mouse (Han, E.D. et al. J. Clin. Invest. 109: 1057-1063, 2002).

Summary

Provided herein are methods for modulating expression of kallikrein mRNA and protein. In certain embodiments, kallikrein specific inhibitors modulate expression of kallikrein mRNA and protein. In certain embodiments, kallikrein specific inhibitors are nucleic acids, proteins, 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, kallikrein mRNA levels are reduced. In certain embodiments, kallikrein protein levels are reduced. In certain embodiments, kallikrein mRNA and protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such diseases, disorders, and conditions are inflammatory conditions. In certain embodiments, the inflammatory condition may be an acute or chronic inflammatory condition. In certain embodiments, such inflammatory conditions may include hereditary angioedema (HAE), edema, angioedema, swelling, angioedema of the lids, ocular edema, macular edema, and cerebral edema.

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 an inflammatory condition include genetic predisposition to an inflammatory condition and environmental factors. In certain embodiments, a defect in an individual's genetic code for complement 1 esterase inhibitor (i.e., C1-INH, a protein that helps to regulate the immune system) is responsible for inflammatory conditions such as hereditary angioedema (HAE). In certain embodiments, genetic mutations lead to a deficiency in C1-INH (i.e., type I HAE) or an inability of existing C1-INH to function properly (i.e., type II HAE). In certain embodiments, genetic mutations in Factor 12 gene lead to hyperfunctionalization of Factor 12, which leads to hereditary angioedema (i.e., type III HAE). In certain embodiments, acquired angioedema may be the result of using angiotensin-converting enzyme inhibitors (i.e., ACE inhibitors) or angiotensin II receptor blockers (i.e., ARBs). In certain embodiments, an allergic reaction may lead to angioedema. Certain outcomes associated with development of an inflammatory condition include edema/swelling in various body parts including the extremities (i.e., hands, feet, arms, legs), the intestines (abdomen), the face, the genitals, the larynx (i.e., voice box); vascular permeability; vascular leakage; generalized inflammation; abdominal pain; bloating; vomiting; diarrhea; itchy skin; respiratory (asthmatic) reactions; rhinitis; anaphylaxis; bronchoconstriction; hypotension; coma; and death.

In certain embodiments, methods of treatment include administering a kallikrein specific inhibitor to an individual in need thereof. In certain embodiments, the kallikrein specific inhibitor is a nucleic acid. In certain embodiments, the nucleic acid is an antisense compound. In certain embodiments, the antisense compound is a modified oligonucleotide.

Detailed Description

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. Additionally, as used herein, the use of "and" 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 disclosure, including, but not limited to, patents, patent applications, published patent applications, articles, books, treatises, and 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 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.

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

"2'-O-methoxyethyl" (also 2'-MOE and 2'-$O(CH_2)_2$-OCH_3) refers to an O-methoxy-ethyl modification of the 2' position of a furanosyl ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

"2'-MOE nucleoside" (also 2'-O-methoxyethyl nucleoside) means a nucleoside comprising a 2'-MOE 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.

"About" means within ±7% of a value. For example, if it is stated, "the compounds affected at least about 70% inhibition of kallikrein", it is implied that the kallikrein levels are inhibited within a range of 63% and 77%.

"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 kallikrein 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" or "ameliorate" or "amerliorating" 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. Examples of antisense compounds include single-stranded and double-stranded compounds, such as, antisense oligonucleotides, siRNAs, shRNAs, snoRNAs, miRNAs, and satellite repeats.

"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 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 furanosyl ring modified by the bridging of two atoms. A bicyclic sugar is a modified sugar.

"Bicyclic nucleoside" (also BNA) 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'-$CH(CH_3)$-O-2'.

"Constrained ethyl nucleoside" (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-$CH(CH_3)$-O-2' bridge.

"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.

"Coagulation factor" means any of factors I, II, III, IV, V, VII, VIII, IX, X, XI, XII, XIII, or TAFI in the blood coagulation cascade. "Coagulation factor nucleic acid" means any nucleic acid encoding a coagulation factor. For example, in certain embodiments, a coagulation factor nucleic acid includes, without limitation, a DNA sequence encoding a coagulation factor (including genomic DNA comprising introns and exons), an RNA sequence transcribed from DNA encoding a coagulation factor, and an mRNA sequence encoding a coagulation factor. "Coagulation factor mRNA" means an mRNA encoding a coagulation factor protein.

"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 a "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.

"Identifying an animal at risk for developing an inflammatory condition" means identifying an animal having been diagnosed with an inflammatory condition or identifying an animal predisposed to develop an inflammatory condition. Individuals predisposed to develop an inflammatory condition include those having one or more risk factors for inflmmatory conditions, including, having a personal or family history of one or more inflammatory conditions. Such identification may be accomplished by any method including evaluating an individual's medical history and standard clinical tests or assessments.

"Identifying an animal at risk for experiencing an attack of hereditary angioedema" means identifying an animal having been diagnosed with hereditary angioedema or identifying an animal predisposed to develop hereditary angioedema. Individuals predisposed to develop hereditary angioedema include those having one or more risk factors for hereditary angioedema, including, having a personal or family history of hereditary angioedema. 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.

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

"Inflammatory condition" or"inflammatory disease" or "inflammatory disorder" or "inflammatory condition" means a disease, disorder or condition related to an inflammatory response to injury or stimulus characterized by clinical signs of increased redness (rubor), temperature (calor), swelling (tumor), pain (dolor) and/or loss of function (functio laesa) in a tissue. Examples of such diseases, disorders, and conditions include hereditary angioedema (HAE).

"Inhibiting kallikrein" means reducing expression of kallikrein mRNA and/or protein levels in the presence of a kallikrein specific inhibitior, including a kallikrein antisense oligonucleotide, as compared to expression of kallikrein mRNA and/or protein levels in the absence of a kallikrein specific inhibitor, such as a kallikrein antisense oligonucleotide.

"Internucleoside linkage" refers to the chemical bond between nucleosides.

"Kallikrein nucleic acid" (aka KLKB1, plasma kallikrein, Fletcher factor, kallikrein B) means any nucleic acid encoding kallikrein. For example, in certain embodiments, a kallikrein nucleic acid includes a DNA sequence encoding kallikrein, an RNA sequence transcribed from DNA encoding kallikrein (including genomic DNA comprising introns and exons), and an mRNA sequence encoding kallikrein. "Kallikrein mRNA" means an mRNA encoding a kallikrein protein.

In certain embodiments, KLKB1 is the term generally associated with the gene. In certain embodiments, the expression product of KLKB1 translation is genally termed plasma prekallikrein. Plasma prekallikrein is cleaved by Factor 12a. In certain embodiments, the cleavage product is generally termed plasma kallikrein. Plasma kallikrein is the substrate that C1-INH acts upon. As used herein, "kallikrein" means KLKB1 and its expression products, including, for example, plasma prekallikrein and plasma kallikrein.

"Kallikrein specific inhibitor" refers to any agent capable of specifically inhibiting the expression of kallikrein mRNA and/or kallikrein protein at the molecular level. For example, kallikrein specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of kallikrein mRNA and/or kallikrein protein. In certain embodiments, by specifically modulating kallikrein mRNA expression and/or kallikrein protein expression, kallikrein specific inhibitors may affect other components of the coagulation cascade including downstream components. Similarly, in certain embodiments, kallikrein specific inhibitors may affect other molecular processes in an animal.

"Linked nucleosides" means adjacent nucleosides which are bonded together.

"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.

"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 derivative" encompasses pharmaceutically acceptable salts, conjugates, prodrugs or isomers of the compounds described herein.

"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" or "preventing" 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," 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" or "treating" 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 occuring 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

Certain embodiments provide methods for decreasing kallikrein mRNA and protein expression.

Certain embodiments provide methods for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with kallikrein in an individual in need thereof. Also contemplated are methods for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with kallikrein. Kallikrein associated diseases, disorders, and conditions include inflammatory conditions. In certain embodiments, the inflammatory condition may be an acute or chronic inflammatory condition. In certain embodiments, such inflammatory conditions include hereditary angioedema (HAE), edema, angioedema, swelling, angioedema of the lids, ocular edema, macular edema, and cerebral edema

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

In certain embodiments of the present invention, kallikrein specific inhibitors are peptides or proteins, such as, but not limited to, lympho-epithelial Kazal-type-related inhibitor (LEKTI) as described in *J Proteome Res* 2010; 9: 4389-4394; ecotin-Pkal as described in *Biol Chem* 2010; 391: 425-433; aprotinin as described in *J Hypertens* 1987; 5: 581-586; PK15 as described in *Nat Chem Biol* 2009; 5: 502-507; kallistatin as described in *Biol Chem* 2001; 382: 15-21 and *J Biol Chem* 1992; 267: 25873-25880; C1-inhibitor as described in *Thromb Haemost* 2004; 92: 1277-1283 and *Adv Biosci* 1978; 17: 93-101; CeKI as described in *Biol Chem* 2004; 385: 1083-1086; AdKi as described in *Toxicon* 2004; 43: 219-223; FE999024 as described in *Am J Pathol* 2001; 159: 1797-1805; Arginine-15-aprotinin as described in *Adv Exp Med Biol* 1989; 247B: 15-21; alpha-1-antitrypsin-Pittsburgh as described in *J Clin Invest* 1986; 77: 631-634; and kallikrein inhibitors as described in US Patent No. 7,235,530, USPPN 2006/0069020, USPPN 2008/0188409, USPPN 2008/0221031, USPPN 2009/0221480, USPPN 2009/0227494, USPPN 2009/0227495, USPPN 2009/0233852, USPPN 2009/0234009, USPPN 2009/0247453, USPPN 2009/0264350, USPPN 2009/0075887; USPPN 2009/0105142, USPPN 2010/0183625, and US Patent No. 4,973,668.

In certain embodiments of the present invention, kallikrein specific inhibitors are antibodies, such as, but not limited to, DX-2300 as described in *Biochem J* 2009; 422: 383-392.

In certain embodiments of the present invention, kallikrein specific inhibitors are small molecules, such as, but not limited to, Ecallantide (DX-88 by Dyax Corp) as described in *Ann Allergy Asthma Immunol* 2010; 105: 430-436 and *Drugs Today* 2010; 46: 547-555; Nafamostat mesilate as described in *J Anesth* 2010; 24: 549-552 and *Br J Aneaesth* 1998; 81: 963-964; CU-2010 as described in *Anesthesiology* 2009; 110: 123-130; VA999024 and VA999026 as described in *Immunopharmacology* 1996; 32: 115-118; PKSI-527 (trans-4-aminomethyl-cyclohexanecarbonylphenylalanine 4-carboxymethylanilide hydrochloride) as described in *Thromb Res* 2005; 116: 403-408; and kallikrein inhibitors as described in US Patent No. 4,153,687.

Certain embodiments provide for methods of treating, preventing, or ameliorating an inflammatory condition in an animal, comprising administering to the animal a therapeutically effective amount of a kallikrein specific inhibitor, wherein the inflammatory condition is

ameliorated in the animal.

In certain embodiments, the animal is a human.

In certain embodiments, the inflammatory condition is hereditary angioedema (HAE).

In certain embodiments, the kallikrein specific inhibitior is an antisense compound. In certain embodiments, the antisense compound is a modified oligonucleotide.

In certain embodiments, the kallikrein specific inhibitor is a nucleic acid. In certain embodiments, the nucleic acid is a modified oligonucleotide.

In certain embodiments, the kallikrein specific inhibitor is a modified oligonucleotide.

In certain embodiments, the modified oligonucleotide consists of 12 to 30 linked nucleosides.

In certain embodiments, the modified oligonucleotide is a single-stranded oligonucleotide.

In certain embodiments, the modified oligonucleotide consists of 15, 16, 17, 18, 19, or 20 linked nucleosides.

In certain embodiments, the modified oligonucleotide has a nucleobase sequence that is 80%, 85%, 90%, 95%, or 100% complementary to a human kallikrein nucleic acid.

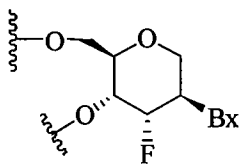
In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage. In certain embodiments, each modified internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, at least one nucleoside of the modified oligonucleotide comprises a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' bridge.

In certain embodiments, the modified sugar comprises a 2'-O-methoxyethyl group.

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

In certain embodiments, at least one nucleoside of the modified oligonucleotide comprises 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.

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In certain embodiments, the modified oligonucleotide of the compound comprises:
(i) a gap segment consisting of linked deoxynucleosides;
(ii) a 5' wing segment consisting of linked nucleosides;
(iii) 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 some such embodiments, each cytosine in the modified oligonucleotide is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide of the compound comprises:
(i) a gap segment consisting of ten linked deoxynucleosides;
(ii) a 5' wing segment consisting of five linked nucleosides;
(iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to 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. In some such embodiments, each cytosine in the modified oligonucleotide is a 5-methylcytosine.

Certain embodiments provide the use of kallikrein specific inhibitors as described herein in the manufacture of a medicament for treating, ameliorating, or preventing an inflammatory condition such as hereditary angioedema.

Certain embodiments provide the use of a kallikrein specific inhibitor as described herein in the manufacture of a medicament for treating, preventing, or ameliorating an inflammtory condition as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Certain embodiments provide a kit for treating, preventing, or ameliorating an inflammatory condition as described herein wherein the kit comprises:
(i) a kallikrein specific inhibitor as described herein; and alternatively
(ii) an additional agent or therapy as described herein.

A kit may further include instructions for using the kit to treat, prevent, or ameliorate an inflammatory condition as described herein by combination therapy as described herein.

In certain embodiments, provided is a compound comprising a modified oligonucleotide. In certain embodiments, the compound comprises a modified oligonucleotide consisting of 12 to 30 linked nucleosides.

In certain embodiments, the modified oligonucleotide targets a kallikrein nucleic acid. In certain embodiments, the kallikrein nucleic acid may be selected from, but is not limited to, one or more of GENBANK Accession No. NM_000892.3 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. DC412984.1 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. CN265612.1 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. AK297672.1 (incorporated herein as SEQ ID NO: 4), GENBANK Accession No. DC413312.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. AV688858.2 (incorporated herein as SEQ ID NO: 6), GENBANK Accession No. CD652077.1 (incorporated herein as SEQ ID NO: 7), GENBANK Accession No. BC143911.1 (incorporated herein as SEQ ID NO: 8), GENBANK Accession No. CB162532.1 (incorporated herein as SEQ ID NO: 9), the complement of GENBANK Accession No. NT_016354.19 truncated from nucleobases 111693001 to 111730000 (incorporated herein as SEQ ID NO: 10), GENBANK Accession No. NM_008455.2 (incorporated herein as SEQ ID NO: 11), GENBANK Accession No. BB598673.1 (incorporated herein as SEQ ID NO: 12), the complement of GENBANK Accession No. NT_039460.7 truncated from nucleobases 6114001 to 6144000 (incorporated herein as SEQ ID NO: 13), GENBANK Accession No. NM_012725.2 (incorporated herein as SEQ ID NO: 14), the complement of GENBANK Accession No. NW_047473.1 truncated from nucleobases 10952001 to 10982000 (incorporated herein as SEQ ID NO: 15), GENBANK Accession No. 3763_123_A (incorporated herein as SEQ ID NO: 16), GENBANK Accession No. XM_002804276.1 (incorporated herein as SEQ ID NO: 17), the complement of GENBANK Accession No. NW_001118167.1 truncated from nucleobases 2358000 to 2391000 (incorporated herein as SEQ ID NO: 18), and GENBANK Accession No. 3804_126_A (incorporated herein as SEQ ID NO: 19).

In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, the complement of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, the complement of SEQ ID NO: 13, SEQ ID NO: 14, the complement of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, the complement of SEQ ID NO: 18, or SEQ ID NO: 19.

In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of a human sequence.

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In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10.

In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10.

In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of a human sequence. In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is 100% complementary to a nucleobase sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10.

Certain embodiments provide a method comprising, (1) identifying an animal at risk for experiencing an attack of hereditary angioedema; and (2) prophylactically administering to the at risk animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a Facter 12 nucleic acid.

Certain embodiments providea method comprising, (1) identifying an animal at risk for developing an inflammatory condition; and (2) prophylactically administering to the at risk animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a Facter 12 nucleic acid.

In certain embodiments, expression of kallikrein mRNA is reduced.

In certain embodiments, expression of kallikrein protein is reduced.

In certain embodiments, the inflammatory condition is an acute inflammatory condition.

In certain embodiments, the acute inflammatory condition is hereditary angioedma.

In certain embodiments, the prophylactic administering of a modified oligonucleotide prevents edema.

In certain embodiments, the prophylactic administering of a modified oligonucleotide prevents vascular permeability.

In certain embodiments, the prophylactic administering of a modified oligonucleotide prevents vascular leakage.

In certain embodiments, the prophylactic administering of a modified oligonucleotide prevents inflammation.

Certain embodiments provide a method comprising prophylactically treating an inflammatory condition in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

In certain embodiments, the inflammatory condition is an acute inflammatory condition.

In certain embodiments, the acute inflammatory condition is hereditary angioedma.

Certain embodiments provide a method comprising inhibiting edmea in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

Certain embodiments provide a method comprising inhibiting vascular permeability in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

Certain embodiments provide a method comprising inhibiting vascular leakage in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

Certain embodiments provide a method comprising inhibiting inflammation in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

In certain embodiements, the animal is a human.

In certain embodiments, the kallikrein nucleic acid is a human kallikrein nucleic acid.

In certain embodiments, the human kallikrein nucleic acid is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10.

In certain embodiments, the modified oligonucleotide is 100% complementary to a human kallikrein nucleic acid.

In certain embodiments, the modified oligonucleotide is a single-stranded oligonucleotide.

In certain embodiments, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

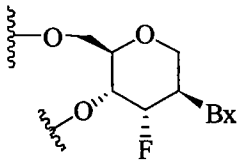
In certain embodiments, the modified oligonucleotide comprises at least one nucleoside having a modified sugar.

In certain embodiments, the modified sugar is a bicyclic sugar.

In certain embodiments, the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' bridge.

In certain embodiments, the modified oligonucleotide comprises 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, the modified sugar comprises a 2'-O-methoxyethyl group.

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 is co-administered with any of the group selected from a serine protease inhibitor C1-INH recombinant protein, Factor 12 antisense oligonucleotide, CINRYZE, BERINERT, KALBITOR, Icatibant, Ecallantide, attenuated androgens, anabolic steroids, and antifibrinolytic agents (e.g., epsilon-aminocaproic acid and tranexamic acid).

Certain embodiments provide, a modified oligonucleotide consisting of 12 to 30 linked nucleosides fully complementary to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4,

SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10 for use in the treatment of an inflammatory condition.

In certain embodiments, the inflammatory condition is hereditary angioedema.

Certain embodiments provide use of a modified oligonucleotide as described herein in the manufacture of a medicament for treating an inflammatory condition.

In certain embodiments, the administering is parenteral administration. In certain embodiments, the parenteral administration is any of subcutaneous or intravenous administration.

Certain embodiments provide a method comprising, increasing or stabilizing HMWK in an animal in need thereof by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

Certain embodiments provide a method of treating an inflammatory condition in an animal in need thereof by increasing or stabilizing HMWK in the animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

In certain embodiments, the inflammatory condition is associated with low levels of HMWK.

In certain embodiments, the inflammatory condition is associated with high levels of bradykinin.

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 is 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 kallikrein 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 nucleosides.

In certain embodiments antisense oligonucleotides targeted to a kallikrein 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 kallikrein 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 kallikrein 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-CH_3, 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 to 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 kallikrein nucleic acid possess a 5-10-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a kallikrein nucleic acid possess a 3-14-3 gapmer motif.

In certain embodiments, antisense compounds targeted to a kallikrein nucleic acid possess a 2-13-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a kallikrein nucleic acid possess a 5-8-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a kallikrein nucleic acid possess a 6-8-6 gapmer motif.

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

In certain embodiments, a gap-widened antisense oligonucleotide targeted to a kallikrein nucleic acid has a gap segment of fourteen 2'-deoxyribonucleotides positioned immediately adjacent

to and between wing segments of three chemically modified nucleosides. In certain embodiments, the chemical modification comprises a 2'-sugar modification. In another embodiment, the chemical modification comprises a 2'-MOE sugar modification.

In certain embodiments, a gap-widened antisense oligonucleotide targeted to a kallikrein nucleic acid has a gap segment of thirteen 2'-deoxyribonucleotides positioned immediately adjacent to and between a 5' wing segment of two chemically modified nucleosides and a 3' wing segment of five chemically modified nucleosides. In certain embodiments, the chemical modification comprises a 2'-sugar modification. In another embodiment, the chemical modification comprises a 2'-MOE sugar modification.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode kallikrein include, without limitation, the following: GENBANK Accession No. NM_000892.3 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. DC412984.1 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. CN265612.1 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. AK297672.1 (incorporated herein as SEQ ID NO: 4), GENBANK Accession No. DC413312.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. AV688858.2 (incorporated herein as SEQ ID NO: 6), GENBANK Accession No. CD652077.1 (incorporated herein as SEQ ID NO: 7), GENBANK Accession No. BC143911.1 (incorporated herein as SEQ ID NO: 8), GENBANK Accession No. CB162532.1 (incorporated herein as SEQ ID NO: 9), the complement of GENBANK Accession No. NT_016354.19 truncated from nucleobases 111693001 to 111730000 (incorporated herein as SEQ ID NO: 10), GENBANK Accession No. NM_008455.2 (incorporated herein as SEQ ID NO: 11), GENBANK Accession No. BB598673.1 (incorporated herein as SEQ ID NO: 12), the complement of GENBANK Accession No. NT_039460.7 truncated from nucleobases 6114001 to 6144000 (incorporated herein as SEQ ID NO: 13), GENBANK Accession No. NM_012725.2 (incorporated herein as SEQ ID NO: 14), the complement of GENBANK Accession No. NW_047473.1 truncated from nucleobases 10952001 to 10982000 (incorporated herein as SEQ ID NO: 15), GENBANK Accession No. 3763_123_A (incorporated herein as SEQ ID NO: 16), GENBANK Accession No. XM_002804276.1 (incorporated herein as SEQ ID NO: 17), the complement of GENBANK Accession No. NW_001118167.1 truncated from nucleobases 2358000 to 2391000 (incorporated herein as SEQ ID NO: 18), and GENBANK Accession No. 3804_126_A (incorporated herein as SEQ ID NO: 19).

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 kallikrein 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 emodiments, 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 preceeding 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 specifcally 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 kallikrein mRNA levels are indicative of inhibition of kallikrein expression. Reductions in levels of a kallikrein protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of kallikrein expression. For example, reduced or prevented inflammation can be indicative of inhibition of kallikrein expression. In another example, reduced or prevented edema/swelling can be indicative of inhibition of kallikrein expression. In another example, reduced or prevented vascular permeability can be indicative of inhibition of kallikrein expression. In another example, reduced or prevented vascular leakage can be indicative of inhibition of kallikrein expression. In certain embodiments, vascular permeability is measured by quanification of a dye, such as Evans Blue.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a kallikrein 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 kallikrein nucleic acid.

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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 kallikrein nucleic acid).

Non-complementary nucleobases between an antisense compound and a kallikrein 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 kallikrein 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 kallikrein 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 4 (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 kallikrein 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 kallikrein 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 kallikrein 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 occuring 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 kallikrein 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 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 chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substitutent 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 $C(R_1)(R_2)$ (R, R_1 and R_2 are each independently H, C_1-C_{12} 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) or 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'-OCH_3, 2'-OCH_2CH_3, 2'-OCH_2CH_2F and 2'-$O(CH_2)_2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH_2)_2SCH_3$, $O(CH_2)_2$-O-$N(R_m)(R_n)$, O-CH_2-C(=O)-$N(R_m)(R_n)$, and O-CH_2-C(=O)-$N(R_l)$-$(CH_2)_2$-$N(R_m)(R_n)$, where each R_l, R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} 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 comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' and 4'-$CH(CH_2OCH_3)$-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH_2-$N(OCH_3)$-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH_2-O-$N(CH_3)$-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-$C(H)(CH_3)$-2' (see Chattopadhyaya *et al., J. Org. Chem.,* 2009, *74*, 118-134); and 4'-CH_2-C-$(=CH_2)$-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

Further reports related to bicyclic nucleosides can also be found in published literature (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.*, 2007, *129(26)* 8362-8379; Elayadi *et al., Curr. Opinion Invest. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; and Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 7,034,133; 7,053,207; 7,399,845; 7,547,684; and 7,696,345; U.S. Patent Publication No. US2008-0039618; US2009-0012281; U.S. Patent Serial Nos. 60/989,574; 61/026,995; 61/026,998; 61/056,564; 61/086,231; 61/097,787; and 61/099,844; Published PCT International applications WO 1994/014226; WO 2004/106356; WO 2005/021570; WO 2007/134181; WO 2008/150729; WO 2008/154401; and WO 2009/006478. 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(=O)-, -C(=NR_a)-, -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)$_2$-J_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_a$$R_b$)-N(R)-O- or –C($R_a$$R_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, and (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.

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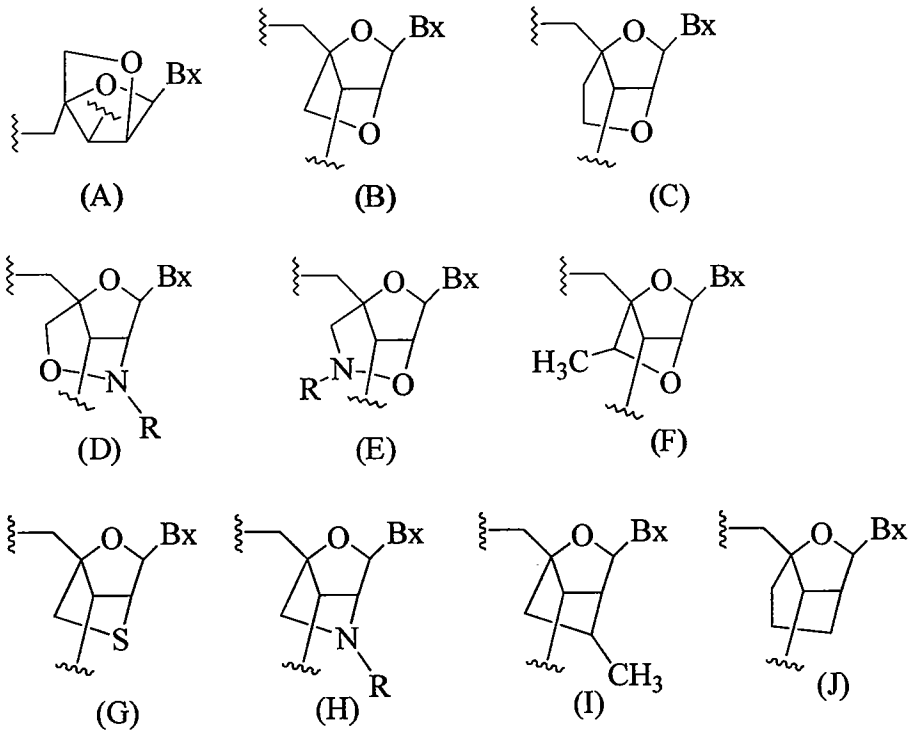
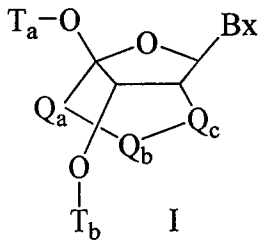


wherein Bx is the base moiety and R is independently H, a protecting group or C_1-C_{12} alkyl.

In certain embodiments, bicyclic nucleosides are provided having Formula I:



I

wherein:

Bx is a heterocyclic base moiety;

$-Q_a-Q_b-Q_c-$ is $-CH_2-N(R_c)-CH_2-$, $-C(=O)-N(R_c)-CH_2-$, $-CH_2-O-N(R_c)-$, $-CH_2-N(R_c)-O-$ or $-N(R_c)-O-CH_2$;

R_c is C_1-C_{12} 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 nucleosides are provided having Formula II:

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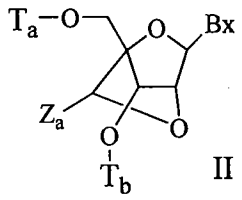


II

wherein:

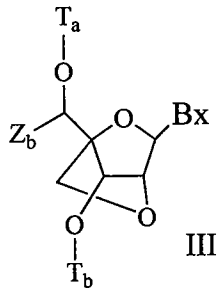
Bx is a heterocyclic base moiety;

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 nucleosides are provided having Formula III:



III

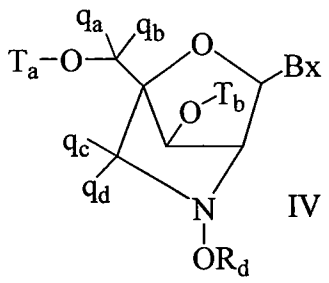
wherein:

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 nucleosides are provided having Formula IV:



IV

wherein:

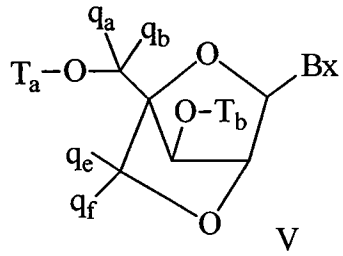
Bx is a heterocyclic base moiety;

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 alkoxyl, substituted C_1-C_6 alkoxyl, acyl, substituted acyl, C_1-C_6 aminoalkyl or substituted C_1-C_6 aminoalkyl;

In certain embodiments, bicyclic nucleosides are provided having Formula V:



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, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ 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.

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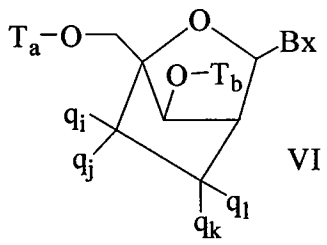
The synthesis and preparation of the methyleneoxy (4'-CH_2-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin *et al.*, *Tetrahedron*, 1998, *54*, 3607-3630). BNAs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

Analogs of methyleneoxy (4'-CH_2-O-2') BNA and 2'-thio-BNAs, have also been prepared (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 (Wengel *et al.*, WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel comformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh *et al.*, *J. Org. Chem.*, 1998, *63*, 10035-10039). In addition, 2'-amino- and 2'-methylamino-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 nucleosides are provided having Formula VI:



VI

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;

each q_i, q_j, q_k and q_l is, independently, H, 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} alkoxyl, substituted C_1-C_{12} alkoxyl, 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 $N(H)C(=S)NJ_jJ_k$; and

q_i and q_j or q_l and q_k together are $=C(q_g)(q_h)$, wherein q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl or substituted C_1-C_{12} alkyl.

One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH_2-2' have been described (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 (Srivastava *et al., J. Am. Chem. Soc.*, 2007, *129(26)*, 8362-8379).

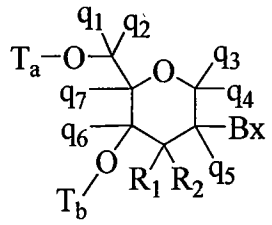
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

As used herein, "monocylic 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: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, 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, F, 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, or a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain (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 (Martin, *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, *Bioorg. Med. Chem.*, 2002, *10*, 841-854), fluoro HNA (F-HNA) or those compounds having Formula VII:



VII

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

Bx is a heterocyclic base moiety;

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

q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each independently, H, 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; and each of R_1 and R_2 is selected from hydrogen, hydroxyl, halogen, subsitituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$ and CN, wherein X is O, S or NJ_1 and each J_1, J_2 and J_3 is, independently, H or C_1-C_6 alkyl.

In certain embodiments, the modified THP nucleosides of Formula VII are provided wherein q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is other than H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is methyl. In certain embodiments, THP nucleosides of Formula VII are provided wherein one of R_1 and R_2 is fluoro. In certain embodiments, R_1 is fluoro and R_2 is H; R_1 is methoxy and R_2 is H, and R_1 is H and R_2 is methoxyethoxy.

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-C_1-C_{10} alkyl, -OCF_3, O-$(CH_2)_2$-O-CH_3, 2'-O$(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-N$(R_m)(R_n)$, or O-CH_2-C(=O)-N$(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. 2'-modifed 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'-OCH_3" or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an -OCH_3 group at the 2' position of the sugar ring.

As used herein, "MOE" or "2'-MOE" or "2'-$OCH_2CH_2OCH_3$" or "2'-O-methoxyethyl" each refers to a nucleoside comprising a sugar comprising a -$OCH_2CH_2OCH_3$ 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 for example review article: Leumann, *Bioorg. Med. Chem.*, 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 nucleosides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleosides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a bicyclic nucleoside having a (4'-$CH(CH_3)$-O-2') bridging group. In certain embodiments, the (4'-$CH(CH_3)$-O-2') modified nucleosides are arranged throughout the wings of a gapmer motif.

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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 kallikrein 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 kallikrein 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 kallikrein nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commerical 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, HepG2 cells, Hep3B cells, 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.

In general, cells are 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 are mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that typically ranges 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 typically ranges 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 are typically 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 kallikrein 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 quantitaive 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.

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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 are 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 (Invetrogen, 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 kallikrein 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 kallikrein nucleic acids can be assessed by measuring kallikrein protein levels. Protein levels of kallikrein 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 kallikrein 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 kallikrein and produce phenotypic changes, such as, reduced inflammation, edema/swelling, vascular permeability, and vascular leakage. In certain embodiments, inflammation is measured by measuring the increase or decrease of edema, temperature, pain, color of tissue, and abdominal function in the animal. 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 kallikrein nucleic acid expression are measured.

Certain Indications

In certain embodiments, the invention provides methods of treating an individual comprising administering one or more pharmaceutical compositions of the present invention. In certain embodiments, the individual has an inflammatory condition. In certain embodiments, the individual is at risk for developing an inflammatory condition, including, but not limited to, hereditary angioedema (HAE), edema, angioedema, swelling, angioedema of the lids, ocular edema, macular edema, and cerebral edema. This includes individuals with an acquired problem, disease, or disorder that leads to a risk of inflammation, for example, genetic predisposition to an inflammatory condition, environmental factors, and exposure to certain medications, including, for example, ACE inhibitors and ARBs. In certain embodiments, the individual has been identified as in need of anti-inflammation therapy. Examples of such individuals include, but are not limited to those having a mutation in the genetic code for complement 1 esterase inhibitor (i.e., C1-INH) or Factor 12. In certain embodiments, an abnormal code can lead to a deficiency in C1-INH (i.e., type I HAE), an inability of existing C1-INH to function properly (type II HAE), or hyperfunctional Factor 12 (i.e.,

type III HAE). In certain embodiments the invention provides methods for prophylactically reducing Factor 12 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 Factor 12 nucleic acid.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to a kallikrein nucleic acid is accompanied by monitoring of kallikrein 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 kallikrein nucleic acid results in reduction of kallikrein 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 kallikrein nucleic acid results in a change in a measure of inflammation, swelling, hypertension, and/or vascular permeability. In certain embodiments, administration of a kallikrein antisense compound increases the measure 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 some embodiments, administration of a kallikrein antisense compound decreases the measure 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 kallikrein are used for the preparation of a medicament for treating a patient suffering or susceptible to an inflammatory condition including hereditary angioedema (HAE).

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, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include anticoagulant or antiplatelet agents. In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include serine protease inhibitor C1-INH recombinant protein, Factor 12 antisense oligonucleotide, CINRYZE, BERINERT, KALBITOR, Icatibant, Ecallantide, attenuated androgens, anabolic steroids, and antifibrinolytic agents (e.g., epsilon-aminocaproic acid and tranexamic acid).

In certain embodiments, pharmaceutical agents that may be co-administered with a kallikrein specific inhibitor of the present invention include, but are not limited to, an additional kallikrein inhibitor. In certain embodiments, the co-adminstered pharmaceutical agent is administered prior to administration of a pharmaceutical composition of the present invention. In certain embodiments, the co-administered pharmaceutical agent is administered following administration of a pharmaceutical composition of the present invention. In certain embodiments the co-administered pharmaceutical agent is administered at the same time as a pharmaceutical composition of the present invention. In certain embodiments the dose of a co-administered pharmaceutical agent is the same as the dose that would be administered if the co-administered pharmaceutical agent was administered alone. In certain embodiments the dose of a co-administered pharmaceutical agent is lower than the dose that would be administered if the co-

administered pharmaceutical agent was administered alone. In certain embodiments the dose of a co-administered pharmaecutical agent is greater than the dose that would be administered if the co-administered pharmaceutical agent was administered alone.

In certain embodiments, the co-administration of a second compound enhances the anti-inflammatory effect of a first compound, such that co-administration of the compounds results in an anti-inflammatory effect that is greater than the effect of administering the first compound alone. In other embodiments, the co-administration results in anti-inflammatory effects that are additive of the effects of the compounds when administered alone. In certain embodiments, the co-administration results in anti-inflammatory effects that are supra-additive of the effects of the compounds when administered alone. In certain embodiments, the first compound is an antisense compound. In certain embodiments, the second compound is an antisense compound.

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: Antisense inhibition of murine kallikrein (KLKB1) mRNA

Antisense oligonucleotides targeting a murine kallikrein nucleic acid were tested for their effects on kallikrein mRNA *in vitro*. Cultured mouse primary hepatocytes at a density of 10,000 cells per well were transfected using Cytofectin reagent with 12.5 nM, 25 nM, 50 nM, 100 nM, or 200 nM of antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and mouse kallikrein mRNA levels were measured by quantitative real-time PCR using the murine primer probe set RTS3313 (forward sequence TGCCTGCTGTTCAGCTTTCTC, designated herein as SEQ ID NO: 21; reverse sequence TGGCAAAGTCCCTGTAATGCT, designated herein as SEQ ID NO: 22; probe sequence CGTGACTCCACCCAAAGAGACAAATAAACG, designated herein as SEQ ID NO: 23).

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Kallikrein mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN.

The chimeric antisense oligonucleotides 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'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. Results demonstrate that kallikrein mRNA levels were significantly reduced in a dose dependent manner.

ISIS 482584 (GGCATATTGGTTTTTGGAAT; incorporated herein as SEQ ID NO: 40), which was one of the antisense oligonucleotides tested in the assay, is targeted to SEQ ID NO: 11 (GENBANK Accession No. NM_008455.2) with a target start site of 1586 and a target stop site of 1605. ISIS 482584 reduced kallikrein mRNA in a dose dependent manner yielding a half maximal inhibitory concentration (IC_{50}) of 84 nM.

Table 1
Dose-dependent antisense inhibition of murine kallikrein

Dose	% inhibition
12.5 nM	0
25.0 nM	36
50.0 nM	17
100.0 nM	60
200.0 nM	83

C57BL/6J-Tyrc-2J mice were treated with 2.5 mg/kg, 5.0 mg.kg, 10.0 mg/kg, 20.0 mg/kg, 40.0 mg/kg, or 80.0 mg/kg (corresponding to 5.0 mg/kg, 10.0 mg/kg, 20.0 mg/kg, 40 mg/kg, 80 mg/kg, or 160 mg/kg mg/kg per week) of ISIS 482584 administered subcutaneously twice a week for 3 weeks. Kallikrein mRNA and protein expression was reduced in a dose dependent manner. Kallikrein mRNA was reduced by greater than 90% inhibition at a dose of 160 mg/kg per week.

Example 2: *In vivo* effect of antisense inhibition of murine kallikrein (KLKB1) in an angioedema mouse model

Hereditary angioedema (HAE) is characterized by local swelling and increase in vascular permeability in subcutaneous tissues (Morgan, B.P. N. Engl. J. Med. 363: 581-83, 2010). It is caused by a deficiency of the C1 inhibitor, a protein of the complement system. Two mouse models were used in this study, including, an established mouse model of C1-INH deficiency and a captopril-induced edema model, both of which casue vascular permeability, a hallmark of HAE. Reversal of vascular permeability is accompanied by increased plasma levels of high molecular weight kininogen (HMWK).

In the first model, angioedema was induced by treatment with Captopril, a known antihypertensive agent, which increases vascular permeability in mice and replicates the pathology of hereditary angioedema.

In the second model, angioedema was induced by treatment with ISIS 461756, an antisense oligonucleotide which targets murine C1 inhibitor mRNA, which increases vascular permeability in mice and replicates the pathology of hereditary angioedema. ISIS 461756 (SEQ ID NO: 41; AAAGTGGTTGATACCCTGGG) is a 5-10-5 MOE gapmer targeting nucleosides 1730-1749 of NM_009776.3 (SEQ ID NO: 20).

The effect of HOE-140 and ISIS 482584, an antisense oligonucleotide inhibitor of kallikrein, were evaluated in the Captopril and ISIS 461756-induced mouse models of vascular permeability. Some of the murine groups were treated with HOE-140, a selective antagonist of the bradykinin B2 receptor, which blocks vasodilation and vascular permeability (Cruden and Newby, Expert Opin. Pharmacol. 9: 2383-90, 2008). Other mice were treated with ISIS 482584, which inhibits kallikrein mRNA expression. The effect of treatment with HOE-140 was compared with the effect of treatment with ISIS 482584.

Treatment

The various treatment groups for this assay are presented in Table 2.

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Group 1 consisted of 4 C57BL/6J-Tyrc-2J mice treated with PBS administered subcutaneously twice a week for 4 weeks. No other treatment was administered to Group 1 which served as a control group to measure the basal level of vascular permeability.

Group 2 consisted of 8 C57BL/6J-Tyrc-2J mice treated with PBS administered subcutaneously twice a week for 4 weeks. At the end of the treatment, the mice were intraperitoneally administered 20 μg of captopril. Group 2 served as a PBS control group for captopril-induced vascular permeability.

Group 3 consisted of 8 C57BL/6J-Tyrc-2J mice treated with PBS administered subcutaneously twice a week for 4 weeks. On day 14, the mice were treated with 50 mg/kg of the antisense oligonucleotide targeting C1 inhibitor, ISIS 461756, administered subcutaneously twice a week for 2 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 3 served as a PBS control group for captopril and ISIS 461756-induced vascular permeability.

Group 4 consisted of 8 C57BL/6J-Tyrc-2J mice treated with PBS administered subcutaneously twice a week for 4 weeks. On day 14, the mice were treated with 50 mg/kg of the antisense oligonucleotide targeting C1 inhibitor, ISIS 461756, administered subcutaneously twice a week for 2 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. The mice were then also intraperitoneally administered 30 μg of HOE-140. Group 4 served as a positive control for inhibition of vascular permeability with HOE-140.

Group 5 consisted of 8 C57BL/6J-Tyrc-2J mice treated with 40 mg/kg of control oligonucleotide ISIS 141923, a 5-10-5 MOE gapmer with no known murine target, (CCTTCCCTGAAGGTTCCTCC; SEQ ID NO: 42) administered subcutaneously twice a week for 4 weeks. On day 14, the mice were treated with 50 mg/kg of the antisense oligonucleotide targeting C1 inhibitor, ISIS 461756, administered subcutaneously twice a week for 2 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 5 served as a control group for captopril and ISIS 461756-induced vascular permeability.

Group 6 consisted of 8 C57BL/6J-Tyrc-2J mice and was treated with 40 mg/kg of ISIS 482584 administered subcutaneously twice a week for 4 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 6 served as the experimental

treatment group for examining the effect of kallikrein ASO on captopril-induced vascular permeability.

Group 7 consisted of 8 C57BL/6J-Tyrc-2J mice treated with 40 mg/kg of ISIS 482584 administered subcutaneously twice a week for 4 weeks. On day 14, the mice were treated with 50 mg/kg of the antisense oligonucleotide targeting C1 inhibitor, ISIS 461756, administered subcutaneously twice a week for 2 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 7 served as the experimental treatment group for examining the effect of kallikrein ASO on captopril and ISIS 461756-induced vascular permeability.

All the groups were then injected with 30 mg/kg of Evans Blue solution into the tail vein. The mice were sacrificed 30 min after the Evans Blue solution administration and colons, feet, ears, and intestines were harvested. Blood samples were taken through cardiac puncture.

Table 2
Treatment groups

Group No.	Treatment	Captopril	ISIS 461756	HOE-140
1. (N=4)	PBS	No	No	No
2. (N=8)	PBS	Yes	No	No
3. (N=8)	PBS	Yes	Yes	No
4. (N=8)	PBS	Yes	Yes	Yes
5. (N=8)	ISIS 141923	Yes	Yes	No
6. (N=8)	ISIS 482584	Yes	No	No
7. (N=8)	ISIS 482584	Yes	Yes	No

Quantification of vascular permeability

The harvested tissues from the feet, colon, ears, and intestines were placed separately in formamide solution overnight to leach out the Evans Blue dye. The formamide solution containing ear and feet tissue was heated to 55^0C and left overnight. The color intensity of the dye-infused formamide solution was then measured at OD_{600nm}, and is presented in Table 3. Mice displaying any manifestation of angioedema take up more dye and, therefore, demonstrate high OD values.

As presented in Table 3, treatment with ISIS 482584 prevents vascular permeability in mice treated with captopril (Group 6) and in mice treated with captopril and ISIS 461756 (Group 7)

compared to the respective PBS control groups (Groups 2 and 3). Measures of vascular permeability in mice of Groups 6 and 7 were also reduced in most of the tissues in comparison to the mice treated with the control oligonucleotide, ISIS 141923 (Group 5), where vascular permeability was induced with captopril and ISIS 461756. Measures of vascular permeability in the colon and feet tissues of both the treatment groups (Groups 6 and 7) were comparable to basal levels, as observed in mice treated with only PBS (Group 1). Reduction in vascular permeability in mice treated with ISIS 482584 was comparable to that seen in mice treated with the bradykinin 2 receptor antagonist, HOE140, which served as a positive control in this assay.

Therefore, antisense inhibition of kallikrein mRNA may be beneficial for the treatment and prevention of vascular permeability, which is symptomatic of HAE.

Table 3

OD_{600nm} of Evans Blue dye to measure vascular permeability

Group No.	Treatment	Captopril	ISIS 461756	HOE-140	Colons	Intestines	Feet	Ears
1	PBS	No	No	No	0.26	0.16	0.11	0.02
2	PBS	Yes	No	No	0.49	0.29	0.12	0.07
3	PBS	Yes	Yes	No	0.49	0.34	0.11	0.12
4	PBS	Yes	Yes	Yes	0.14	0.18	0.07	0.09
5	ISIS 141923	Yes	Yes	No	0.44	0.29	0.14	0.08
6	ISIS 482584	Yes	No	No	0.27	0.30	0.07	0.14
7	ISIS 482584	Yes	Yes	No	0.21	0.34	0.07	0.06

Quantification of High Molecular Weight Kininogen (HMWK)

Western blot quantification of HMWK from blood samples are presented in Figure 1.

As shown in Figure 1, samples from Groups 1 and 2 have low levels of HMWK as compared to Groups 6 and 7 indicating that vascular permeability is reversed in Groups 6 and 7. Also as shown in Figure 1, samples from Groups 1 and 2 have increased HMWK cleavage product as compared to Groups 6 and 7. Thus, lack of HMWK is caused by kallikrein cleavage of HMWK into cleavage products (including bradykinin and HKa).

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Example 3: *In vivo* effect of antisense inhibition of murine kallikrein (KLKB1) on basal permeability and captopril-induced permeability in mice

Basal permeability is the level of vascular permeability occurring in the tissues of naïve, untreated mice. The effect of ISIS 482584 in the prevention of vascular permeability, either basal or captopril-induced, was evaluated.

Treatment

The various treatment groups for this assay are presented in Table 4.

Group 1 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 4 weeks. No other treatment was administered to Group 1 which served as a control group to measure the basal levels of vascular permeability.

Group 2 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 4 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 2 served as the negative control group for captopril-induced vascular permeability.

Group 3 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 4 weeks. At the end of the treatment period, the mice were intraperitoneally administered 30 μg of HOE-140. Group 3 served as a positive control for inhibition of basal vascular permeability.

Group 4 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 4 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. The mice were also intraperitoneally administered 30 μg of HOE-140. Group 4 served as a positive control for inhibition of captopril-induced vascular permeability.

Group 5 consisted of 8 mice and was treated with 40 mg/kg of ISIS 482584 administered subcutaneously twice a week for 4 weeks. Group 5 served as an experimental treatment group for examining the effect of ISIS 482584 on basal vascular permeability.

Group 6 consisted of 8 mice and was treated with 40 mg/kg of ISIS 482584 administered subcutaneously twice a week for 4 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 6 served as an experimental treatment group for examining the effect of ISIS 482584 on captopril-induced vascular permeability.

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All the groups were then injected with 30 mg/kg of Evans Blue solution. The mice were sacrificed 30 min after the Evans Blue solution administration and colons, feet, ears, and intestines were harvested.

Table 4
Treatment groups

Group No.	Treatment	Captopril	HOE-140
1. (N=8)	PBS	No	No
2. (N=8)	PBS	Yes	No
3. (N=8)	PBS	No	Yes
4. (N=8)	PBS	Yes	Yes
5. (N=8)	ISIS 482584	No	No
6. (N=8)	ISIS 482584	Yes	No

Quantification of vascular permeability

The harvested tissues from the feet, colon, intestine, and ears were placed separately in formamide solution overnight to leach out the Evans Blue dye. The formamide solution containing feet and ear tissue was heated to 55^0C and left overnight. The color intensity of the dye-infused formamide solution was then measured at OD_{600nm}, and is presented in Table 5. Mice displaying any manifestation of angioedema take up more dye and, therefore, demonstrate high OD values.

As presented in Table 5, mice treated with ISIS 482584 demonstrated reduced basal vascular permeability compared to the PBS control (Group 5 vs. Group 1). The reduction in basal vascular permeability by treatment with ISIS 482584 was comparable to that caused by treatment with HOE-140 (Group 3, which served as the positive control). Mice treated with ISIS 482584 also demonstrated reduced captopril-induced vascular permeability in most tissues compared to the PBS control (Group 6 vs. Group 2). The reduction in captopril-induced vascular permeability by treatment with ISIS 482584 was comparable to that caused by treatment with HOE-140 (Group 4, which served as the positive control).

Table 5
OD_{600nm} of Evans Blue dye to measure vascular permeability

Group No.	Treatment	Captopril	HOE-140	Colon	Feet	Intestine	Ears
1	PBS	No	No	0.27	0.08	0.23	0.06
2	PBS	Yes	No	0.61	0.08	0.24	0.01

3	PBS	No	Yes	0.18	0.06	0.21	0.03
4	PBS	Yes	Yes	0.29	0.03	0.14	0.00
5	ISIS 482584	No	No	0.19	0.07	0.22	0.04
6	ISIS 482584	Yes	No	0.37	0.05	0.22	0.00

Example 4: Dose-dependent effect of antisense inhibition of murine kallikrein (KLKB1) on captopril-induced vascular permeability

The effect of varying doses on ISIS 482584 on captopril-induced vascular permeability was evaluated.

Treatment

The various treatment groups for this assay are presented in Table 6.

Group 1 consisted of 4 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. No other treatment was administered to Group 1 which served as a control group to measure the basal levels of vascular permeability.

Group 2 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 2 served as the control group for captopril-induced vascular permeability.

Group 3 consisted of 4 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. The mice were also intraperitoneally administered 30 μg of Icatibant (HOE-140). Group 4 served as a positive control for inhibition of captopril-induced vascular permeability.

Groups 4, 5, 6, 7, 8, and 9 consisted of 8 mice each and were treated with 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, or 80 mg/kg (corresponding to 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, 80 mg/kg, or 160 mg/kg per week), respectively of ISIS 482584 administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice of all the groups were intraperitoneally administered 20 μg of captopril. Groups 4-9 served as the experimental treatment groups for examining the effect of varying doses of ISIS 482584 on captopril-induced vascular permeability.

All the groups were then injected with 30 mg/kg of Evans Blue solution in the tail vein. The mice were sacrificed 30 min after the Evans Blue solution administration and colons, feet, ears, and intestines were harvested. Blood samples were taken through cardiac puncture.

Table 6

Treatment groups

Group No.	Treatment	Dose (mg/kg/wk)	Captopril	HOE-140
1. (N=4)	PBS	-	No	No
2. (N=8)	PBS	-	Yes	No
3. (N=4)	PBS	-	Yes	Yes
4. (N=8)	ISIS 482584	160	Yes	No
5. (N=8)	ISIS 482584	80	Yes	No
6. (N=8)	ISIS 482584	40	Yes	No
7. (N=8)	ISIS 482584	20	Yes	No
8. (N=8)	ISIS 482584	10	Yes	No
9. (N=8)	ISIS 482584	5	Yes	No

Quantification of vascular permeability

The harvested tissues were placed in formamide solution overnight to leach out the Evans Blue dye. The formamide solution containing feet and ear tissue was heated to 55^0C and left overnight. The color intensity of the dye-infused formamide solution was then measured at OD_{600nm}, and is presented in Table 7. Mice displaying any manifestation of angioedema take up more dye and, therefore, demonstrate high OD values.

As presented in Table 7, mice treated with higher doses of ISIS 482584 (Groups 4, 5, and 6) had reduced levels of captopril-induced vascular permeability compared to the corresponding PBS control group (Group 2). The reduction in vascular permeability in mice of these treatment groups (Groups 4 and 5) was comparable to the levels of basal vascular permeability (as shown in Group 1) as well as in mice treated with HOE-140 (Group 3).

Table 7

OD_{600nm} of Evans Blue dye to measure vascular permeability

Group No.	Treatment	Dose (mg/kg)	Captopril	HOE-140	Colon	Feet	Intestine	Ears
1	PBS	-	No	No	0.16	0.07	0.13	0.01

2	PBS	-	Yes	No	0.39	0.12	0.18	0.07
3	PBS	-	Yes	Yes	0.15	0.03	0.10	0.04
4	ISIS 482584	160	Yes	No	0.26	0.10	0.15	0.05
5	ISIS 482584	80	Yes	No	0.21	0.04	0.17	0.03
6	ISIS 482584	40	Yes	No	0.36	0.10	0.20	0.05
7	ISIS 482584	20	Yes	No	0.40	0.11	0.20	0.07
8	ISIS 482584	10	Yes	No	0.41	0.10	0.19	0.05
9	ISIS 482584	5	Yes	No	0.41	0.10	0.17	0.05

Quantification of vascular leakage

The blood drawn through cardiac puncture was immediately mixed with 3 times the volume of ice-cold ethanol. The solution was centrifuged at 15,000 g for 20 minutes at 4^0C to remove cell debris and precipitated plasma proteins. The ethanol extracts were further purified by ultra-filtration through a 10 kDa MWCO filter. The color intensity of the ethanol extracted plasma solution was then measured at OD_{620nm}. The results are presented in Table 8 as percentage increase or decrease of the OD values of the Group 1 PBS control. It was expected that tissues from mice displaying manifestation of angioedema would leak more dye from the plasma and, therefore, demonstrate low OD values, whereas treatment groups may display higher OD values due to reduced vascular leakage. Mice treated with 160 mg/kg/week and 80 mg/kg/week of ISIS 482584 (Groups 4 and 5) demonstrated less vascular leakage compared to the PBS negative control treated with captopril (Group 2). The results from Groups 4 and 5 were comparable to the positive control treated with HOE-140 (Group 3).

Table 8

Percentage of OD_{620nm} of Evans Blue dye compared to the PBS basal control to measure vascular leakage

Group No.	Treatment	Dose (mg/kg)	Captopril	HOE-140	Plasma
2	PBS	-	Yes	No	-43
3	PBS	-	Yes	Yes	5
4	ISIS 482584	160	Yes	No	91
5	ISIS 482584	80	Yes	No	40
6	ISIS 482584	40	Yes	No	-31
7	ISIS 482584	20	Yes	No	-26
8	ISIS 482584	10	Yes	No	-20
9	ISIS 482584	5	Yes	No	-23

Example 5: Dose-dependent effect of antisense inhibition of murine kallikrein (KLKB1) on basal permeability in mice

The effect of varying doses on ISIS 482584 on basal vascular permeability was evaluated.

Treatment

The various treatment groups for this assay are presented in Table 9.

Group 1 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. No other treatment was administered to Group 1 which served as a control group to measure the basal levels of vascular permeability.

Group 2 consisted of 4 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice were intraperitoneally administered 30 μg of HOE-140. Group 2 served as a positive control for inhibition of basal vascular permeability.

Groups 3, 4, 5, 6, 7, and 8 consisted of 8 mice each and were treated with 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, or 80 mg/kg (corresponding to 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, 80 mg/kg, or 160 mg/kg per week), respectively of ISIS 482584 administered subcutaneously twice a week for 3 weeks. Groups 4-9 served as the experimental treatment groups for examining the effect of varying doses of ISIS 482584 on basal vascular permeability.

All the groups were then injected with 30 mg/kg of Evans Blue solution in the tail vein. The mice were sacrificed 30 min after the Evans Blue solution administration and colons, feet, and ears were harvested and examined for permeability defects. Blood samples were taken through cardiac puncture.

Table 9
Treatment groups

Group No.	Treatment	Dose (mg/kg/week)	HOE-140
1. (N=8)	PBS	-	No
2. (N=4)	PBS	-	Yes
3. (N=8)	ISIS 482584	160	No
4. (N=8)	ISIS 482584	80	No

5. (N=8)	ISIS 482584	40	No
6. (N=8)	ISIS 482584	20	No
7. (N=8)	ISIS 482584	10	No
8. (N=8)	ISIS 482584	5	No

Quantification of vascular permeability

The harvested tissues from the feet, colon, and ears were placed in formamide solution overnight to leach out the Evans Blue dye. The formamide solution containing feet and ear tissue was heated to 55^{0}C and left overnight. The color intensity of the dye-infused formamide solution was then measured at OD_{600nm}, and is presented in Table 10. Higher OD values are associated with higher levels of permeability.

As presented in Table 10, most of the tissues of mice treated with ISIS 482584 at all doses (Groups 3-8) demonstrated reduced basal vascular permeability compared to the PBS control (Group 1). The reduction in basal vascular permeability of the ISIS oligonucleotide-treated groups was comparable to the same demonstrated in the positive control group treated with HOE-140 (Group 2).

Table 10

OD_{600nm} of Evans Blue dye to measure vascular permeability

Group No.	Treatment	Dose (mg/kg/week)	HOE-140	Colon	Feet	Ears
1	PBS	-	No	0.27	0.17	0.013
2	PBS	-	Yes	0.24	0.09	0.047
3	ISIS 482584	160	No	0.25	0.11	0.019
4	ISIS 482584	80	No	0.24	0.09	0.014
5	ISIS 482584	40	No	0.27	0.11	0.011
6	ISIS 482584	20	No	0.26	0.11	0.009
7	ISIS 482584	10	No	0.31	0.10	0.015
8	ISIS 482584	5	No	0.32	0.11	0.009

Quantification of vascular leakage

The blood drawn through cardiac puncture was immediately mixed with 3 times the volume of ice-cold ethanol. The solution was centrifuged at 15,000 g for 20 minutes at 4^{0}C to remove cell

debris and precipitated plasma proteins. The ethanol extracts were further purified by ultra-filtration through a 10 kDa MWCO filter. The color intensity of the ethanol extracted plasma solution was then measured at OD_{620nm}. The results are presented in Table 11 as percentage increase or decrease of the OD values of the Group 1 PBS control. It was expected that treatment groups may display higher OD values due to reduced vascular leakage. All the mice in the ISIS oligonucleotide-treated groups demonstrated significantly reduced vascular leakage compared to the PBS negative control.

Table 11

Percentage of OD_{620nm} of Evans Blue dye compared to the PBS basal control to measure vascular leakage

Group No.	Treatment	Dose (mg/kg/week)	HOE-140	Plasma
2. (N=8)	ISIS 482584	160	No	95
3. (N=8)	ISIS 482584	80	No	93
4. (N=8)	ISIS 482584	40	No	83
5. (N=8)	ISIS 482584	20	No	56
6. (N=8)	ISIS 482584	10	No	36

Quantification of High Molecular Weight Kininogen (HMWK)

Western blot quantification of HMWK from blood samples are presented in Figure 2 and Tables 12 and 13.

As shown in Table 12, Groups treated with 482584 have higher levels of HMWK as compared to PBS control, increasing in a dose-dependent manner. Treatment with kallikrein antisense oligonucleotide results in stabilization of HMWK. Thus, vascular permeability is reduced in ISIS 482584-treated groups in a dose-dependent manner. As shown in Table 13, Groups treated with ISIS 482584 have lower HMWK cleavage product as compared to PBS control, decreasing in a dose-dependent manner. Thus, reduced HMWK is caused by kallikrein cleavage of HMWK into cleavage products (including bradykinin and HKa). Data are presented in Intensity Units as measured by densitometer.

Table 12

Quantification of HMWK by densitometer

Group No	Treatment	Dose (mg/kg/week)	Intensity Units
1	PBS	-	89
3	ISIS 482584	160	21358
4	ISIS 482584	80	7279

5	ISIS 482584	40	873
6	ISIS 482584	20	608
7	ISIS 482584	10	507

Table 13

Quantification of HMWK cleavage product by densitometer

Group No	Treatment	Dose (mg/kg/week)	Intensity Units
1	PBS	-	401738
3	ISIS 482584	160	19936
4	ISIS 482584	80	204482
5	ISIS 482584	40	388135
6	ISIS 482584	20	403360
7	ISIS 482584	10	414774

Example 6: Combination therapy of antisense oligonucleotides targeting kallikrein (KLKB1) and Factor 12 on captopril-induced vascular permeability in mice

Mice were treated varying doses of ISIS 410944, a 5-10-5 MOE gapmer targeting Factor 12 (GCATGGGACAGAGATGGTGC; SEQ ID NO: 43), and ISIS 482584 in a captopril-induced vascular permeability model.

Treatment

The various treatment groups for this assay are presented in Table 14.

Group 1 consisted of 4 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. No other treatment was administered to Group 1 which served as a control group to measure the basal levels of vascular permeability.

Group 2 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice were intraperitoneally administered 20 μg of captopril. Group 2 served as the control group for captopril-induced vascular permeability.

Group 3 consisted of 4 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice were intraperitoneally administered

20 μg of captopril. The mice were also intraperitoneally administered 30 μg of HOE-140. Group 3 served as a positive control for inhibition of captopril-induced vascular permeability.

Groups 4, 5, 6, 7, and 8 consisted of 8 mice each and were treated with 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg (corresponding to 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, or 80 mg/kg per week), respectively of ISIS 482584 and ISIS 410944 each administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice of all the groups were intraperitoneally administered 20 μg of captopril. Groups 4-8 served as the experimental treatment groups for examining the effect of ISIS 410944 and ISIS 482584 on captopril-induced vascular permeability.

All the groups were then injected with 30 mg/kg of Evans Blue solution in the tail vein. The mice were sacrificed 30 min after the Evans Blue solution administration and colons, feet, ears, and intestines were harvested.

Table 14
Treatment groups

Group No.	Treatment	Dose (mg/kg/wk) of each ASO	Captopril	HOE-140
1. (N=4)	PBS	-	No	No
2. (N=8)	PBS	-	Yes	No
3. (N=4)	PBS	-	Yes	Yes
4. (N=8)	ISIS 482584+ISIS 410944	80	Yes	No
5. (N=8)	ISIS 482584+ISIS 410944	40	Yes	No
6. (N=8)	ISIS 482584+ISIS 410944	20	Yes	No
7. (N=8)	ISIS 482584+ISIS 410944	10	Yes	No
8. (N=8)	ISIS 482584+ISIS 410944	5	Yes	No

Quantification of vascular permeability

The harvested tissues from the feet, colon, and ears were placed in formamide solution overnight to leach out the Evans Blue dye. The formamide solution containing feet and ear tissue

was heated to 55^{0}C and left overnight. The color intensity of the dye-infused formamide solution was then measured at OD_{600nm}, and is presented in Table 15. Higher OD values are associated with higher levels of permeability.

As presented in Table 15, most of the tissues of mice treated with a combination of ISIS 482584 and ISIS 410944 at all doses (Groups 3-8) demonstrated reduced vascular permeability compared to the PBS control (Group 1). The reduction in vascular permeability of the ISIS oligonucleotide-treated groups was comparable to the same demonstrated in the basal PBS control (Group 1), as well as the positive control group treated with HOE140 (Group 2). Combination of kallikrein and Factor 12 antisense oligonucleotides results in synergistic decrease in permeability. As expected, a corresponding synergistic decrease in vascular leakage was also observed.

Table 15

OD_{600nm} of Evans Blue dye to measure vascular permeability

Group No.	Treatment	Dose (mg/kg/wk) of each ASO	Captopril	HOE-140	Colon	Feet	Intestines	Ears
1	PBS	-	No	No	0.24	0.11	0.13	0.01
2	PBS	-	Yes	No	0.38	0.15	0.11	0.05
3	PBS	-	Yes	Yes	0.23	0.06	0.15	0.04
4	ISIS 482584+ISIS 410944	80	Yes	No	0.19	0.07	0.11	0.04
5	ISIS 482584+ISIS 410944	40	Yes	No	0.19	0.07	0.12	0.03
6	ISIS 482584+ISIS 410944	20	Yes	No	0.22	0.08	0.12	0.04
7	ISIS 482584+ISIS 410944	10	Yes	No	0.38	0.13	0.13	0.05
8	ISIS 482584+ISIS 410944	5	Yes	No	0.53	0.12	0.13	0.03

Example 7: Combination therapy of antisense oligonucleotides targeting kallikrein (KLKB1) and Factor 12 on basal vascular permeability in mice

Mice were treated with varying doses of ISIS 410944, an antisense oligonucleotide targeting Factor 12, and ISIS 482584 in a basal vascular permeability model.

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Treatment

The various treatment groups for this assay are presented in Table 16.

Group 1 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. No other treatment was administered to Group 1 which served as a control group to measure the basal levels of vascular permeability.

Group 2 consisted of 4 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. At the end of the treatment period, the mice were intraperitoneally administered 30 μg of HOE-140. Group 2 served as a positive control for inhibition of basal vascular permeability.

Groups 3, 4, 5, 6, and 7 consisted of 8 mice each and were treated with 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg (corresponding to 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, or 80 mg/kg per week), respectively of ISIS 482584 and ISIS 410944 each administered subcutaneously twice a week for 3 weeks. Groups 3-7 served as the experimental treatment groups for examining the effect of ISIS 410944 and ISIS 482584 on basal vascular permeability.

All the groups were then injected with 30 mg/kg of Evans Blue solution in the tail vein. The mice were sacrificed 30 min after the Evans Blue solution administration and colons, feet, ears, and intestines were harvested.

Table 16
Treatment groups

Group No.	Treatment	Dose (mg/kg/wk)	HOE-140
1. (N=8)	PBS	-	No
2. (N=4)	PBS	-	Yes
3. (N=8)	ISIS 482584+ISIS 410944	80	No
4. (N=8)	ISIS 482584+ISIS 410944	40	No
5. (N=8)	ISIS 482584+ISIS 410944	20	No
6. (N=8)	ISIS 482584+ISIS 410944	10	No
7. (N=8)	ISIS 482584+ISIS 410944	5	No

Quantification of vascular permeability

The harvested tissues from the feet, colon, intestines, and ears were placed in formamide solution overnight to leach out the Evans Blue dye. The formamide solution containing feet and ear tissue was heated to 55^{0}C and left overnight. The color intensity of the dye-infused formamide solution was then measured at OD_{600nm}, and is presented in Table 17. Higher OD values are associated with higher levels of permeability.

As presented in Table 17, most of the tissues of mice treated with a combination of ISIS 482584 and ISIS 410944 at all doses (Groups 2-7) demonstrated reduced vascular permeability compared to the PBS control (Group 1). The reduction in vascular permeability of the ISIS oligonucleotide-treated groups was comparable to the same demonstrated in positive control group treated with HOE140 (Group 2). Combination of kallikrein and Factor 12 antisense oligonucleotides results in synergistic decrease in permeability. As expected, a corresponding synergistic decrease in vascular leakage was also observed.

Table 17

OD_{600nm} of Evans Blue dye to measure vascular permeability

Group No.	Treatment	Dose (mg/kg/wk)	HOE-140	Colon	Feet	Intestines	Ears
1	PBS	-	No	0.19	0.08	0.10	0.004
2	PBS	-	Yes	0.14	0.04	0.08	0.008
3	ISIS 482584+ISIS 410944	80	No	0.14	0.04	0.09	0.01
4	ISIS 482584+ISIS 410944	40	No	0.15	0.05	0.10	0.006
5	ISIS 482584+ISIS 410944	20	No	0.15	0.04	0.10	0.007
6	ISIS 482584+ISIS 410944	10	No	0.15	0.06	0.10	0.004
7	ISIS 482584+ISIS 410944	5	No	0.14	0.05	0.13	0.002

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Example 8: Inhibition of Factor 12 protein activation by ISIS 482584

The effect of antisense inhibition of kallikrein mRNA on Factor 12 protein activation was evaluated.

Treatment

The various treatment groups for this assay are presented in Table 18.

Group 1 consisted of 8 mice and was treated with PBS administered subcutaneously twice a week for 3 weeks. No other treatment was administered to Group 1 which served as a control group to measure Factor 12 activation.

Groups 2, 3, 4, 5, and 6 consisted of 8 mice each and were treated with 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg (corresponding to 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, or 80 mg/kg per week), respectively of ISIS 482584 administered subcutaneously twice a week for 3 weeks. Groups 2-6 served as the treatment groups for measuring the effect of ISIS 482584 on Factor 12 activation.

At the end of the treatment period, plasma was harvested from the mice for the Spectrozyme® Factor 12a based amidolytic assay for Factor 12 in plasma.

Table 18
Treatment groups

Group No.	Treatment	Dose (mg/kg/wk)
1. (N=8)	PBS	-
2. (N=8)	ISIS 482584	80
3. (N=8)	ISIS 482584	40
4. (N=8)	ISIS 482584	20
5. (N=8)	ISIS 482584	10
6. (N=8)	ISIS 482584	5

Assay for Factor 12 activation in plasma

Plasma (5 μL) was added to 85 μL of PBS with 1ug/ml dextran sulfate (500kDa) in a 96 well polypropelene microplate and the solution was incubated for 5 minutes at room temperature. Spectrozyme® FXIIa (10 μL of a 2 mM solution) and 0.2 mM KALLISTOP™ solution was added

and the absorbance kinetic was measured at 405 nm. Factor 12 activation was measured in the linear phase of absorbance accumulation. The results are presented in Table 19 as a percentage of Factor 12 activation measured in the PBS control sample. As observed in Table 19, inhibition of kallikrein by ISIS 482584 results in decreased activation of Factor 12 by its substrate, implying the that PKK is required for proper factor 12 activation.

Table 19

Percentage Factor 12 activation compared to the PBS control

Dose (mg/kg/wk)	% F12 activation
80	14
40	24
20	47
10	63
5	82

Example 9: *In vivo* effect of antisense inhibition of murine kallikrein (KLKB1) on C1-INH antisense oligonucleotide-induced vascular permeability

Vascular permeability induced by ISIS 461756, an antisense oligonucleotide which targets murine C1 inhibitor mRNA, increases vascular permeability in mice and replicates the pathology of hereditary angioedema. The effect of ISIS 482584 on this model was evaluated.

Treatment

One group of 8 mice was treated with 40 mg/kg ISIS 482584 administered subcutaneously twice a week for 3 weeks (weekly dose of 80 mg/kg). A second group of 8 mice was treated with 40 mg/kg of the control oligonucleotide, ISIS 141923, administered subcutaneously twice a week for 3 weeks (weekly dose of 80 mg/kg). A third group of 8 mice was treated with PBS administered subcutaneously twice a week for 3 weeks. On day 14, all the groups were treated with 12.5 mg/kg ISIS 461756 administered subcutaneously twice a week for 3 weeks (weekly dose of 25 mg/kg). A control group of mice was treated with PBS administered subcutaneously twice a week for 3 weeks but was not administered ISIS 461756.

At the end of the treatment period, all the groups were injected with 30 mg/kg of Evans Blue solution into the tail vein. The mice were sacrificed 30 min after the Evans Blue solution

administration and colons, feet, ears, and intestines were harvested. The liver was also harvested for RNA analysis.

RNA analysis

RNA was isolated from the liver for RT-PCR analysis of C1-INH and kallikrein mRNAs. The primer probe set for C1-INH is RTS3218 (forward sequence GAGTCCCCCAGAGCCTACAGT, designated herein as SEQ ID NO: 24; reverse sequence TGTCATTTGTTATTGTGATGGCTACA, designated herein as SEQ ID NO: 25; probe sequence CTGCCCTCTACCTGGCCAACAACCA, designated herein as SEQ ID NO: 26). The primer probe set for kallikrein is RTS3287 (forward sequence ACAAGTGCATTTTACAGACCAGAGTAC, designated herein as SEQ ID NO: 27; reverse sequence GGTTGTCCGCTGACTTTATGCT, designated herein as SEQ ID NO: 28; probe sequence AAGCACAGTGCAAGCGGAACACCC, designated herein as SEQ ID NO: 29). The results are presented in Table 20 as percent inhibition compared to the PBS control not treated with ISIS 461756. The data indicates that ISIS 461756 significantly reduced C1-INH mRNA expression and that treatment with ISIS 482584 significantly reduced kallikrein expression.

Table 20

Percent inhibition of mRNA expression in mice treated with ISIS 461756 compared to the untreated PBS control

Treatment	C1-INH mRNA	kallikrein mRNA
PBS	76	0
ISIS 141923	79	0
ISIS 482584	77	78

Quantification of vascular permeability

The harvested tissues from the feet, colon, and intestines were placed in formamide solution overnight to leach out the Evans Blue dye. The formamide solution containing feet tissue was heated to 55^0C and left overnight. The color intensity of the dye-infused formamide solution was then measured at OD_{600nm}. The data is presented in Table 21 as percent increase or reduction compared to the PBS control not treated with ISIS 461756. The data indicates that treatment with ISIS 482584 prevented vascular permeability induced by ISIS 461756.

Table 21

Percent change in vascular permeability in mice treated with ISIS 461756 compared to the untreated PBS control

Treatment	Colon	Feet	Intestines
PBS	13	70	27
ISIS 141923	2	80	14
ISIS 482584	-23	2	-25

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CLAIMS

What is claimed is:

1. A method comprising,
identifying an animal at risk for experiencing an attack of hereditary angioedema; and
prophylactically administering to the at risk animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

2. A method comprising,
identifying an animal at risk for developing an inflammatory condition; and
prophylactically administering to the at risk animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

3. The method of claims 1 and 2, wherein expression of kallikrein mRNA is reduced

4. The method of claims 1 and 2, wherein expression of kallikrein protein is reduced.

5. The method of claim 4, wherein the inflammatory condition is an acute inflammatory condition.

6. The method of claim 5, wherein the acute inflammatory condition is hereditary angioedma.

7. The method of any of the preceding claims, wherein the prophylactic administering of a modified oligonucleotide prevents edema.

8. The method of any of the preceding claims, wherein the prophylactic administering of a modified oligonucleotide prevents vascular permeability.

9. The method of any of the preceding claims, wherein the prophylactic administering of a modified oligonucleotide prevents vascular leakage.

10. The method of any of the preceding claims, wherein the prophylactic administering of a modified oligonucleotide prevents inflammation.

11. A method comprising,

prophylactically treating an inflammatory condition in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

12. The method of claim 11, wherein the inflammatory condition is an acute inflammatory condition.

13. The method of claim 12, wherein the acute inflammatory condition is hereditary angioedma.

14. A method comprising,

inhibiting edmea in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

15. A method comprising,

inhibiting vascular permeability in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

16. A method comprising,

inhibiting vascular leakage in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

17. A method comprising,

inhibiting inflammation in an animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

18. The method of any of the preceding claims, wherein the animal is a human.

19. The method of any of the preceding claims, wherein the kallikrein nucleic acid is a human kallikrein nucleic acid.

20. The method of any of the preceding claims, wherein the human kallikrein nucleic acid is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10.

21. The method of any of the preceding claims, wherein the modified oligonucleotide is 100% complementary to a human kallikrein nucleic acid.

22. The method of any of the preceding claims, wherein the modified oligonucleotide is a single-stranded oligonucleotide.

23. The method of any of the preceding claims, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

24. The method of claim 23, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

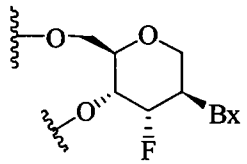
25. The method of any of the preceding claims, comprising at least one nucleoside having a modified sugar.

26. The method of claim 25, wherein the modified sugar is a bicyclic sugar.

27. The method of claim 26, wherein the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' bridge.

28. The method of claim 25, comprising at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

29. The compound of claim 28, wherein each of the at least one tetrahydropyran modified nucleoside has the structure:



wherein Bx is an optionally protected heterocyclic base moiety.

30. The method of claim 25, wherein the modified sugar comprises a 2'-O-methoxyethyl group.

31. The method of any of the preceding claims, wherein at least one nucleoside comprises a modified nucleobase.

32. The method of claim 31, wherein the modified nucleobase is a 5-methylcytosine.

33. The method of any of the preceding claims, comprising co-administering the modified oligonucleotide and any of the group selected from a serine protease inhibitor C1-INH recombinant protein, Factor 12 antisense oligonucleotide, CINRYZE, BERINERT, KALBITOR, Icatibant, Ecallantide, attenuated androgens, anabolic steroids, antifibrinolytic agents, epsilon-aminocaproic acid, tranexamic acid.

34. The method of any of the preceding claims, wherein the administering is parenteral administration.

35. The method of claim 34, wherein the parenteral administration is any of subcutaneous or intravenous administration.

36. A modified oligonucleotide consisting of 12 to 30 linked nucleosides fully complementary to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6,

SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or the complement of SEQ ID NO: 10 use in the treatment of an inflammatory condition.

37. The method of claim 36, wherein the inflammatory condition is hereditary angioedema.

38. Use of a modified oligonucleotide complementary to kallikrein in the manufacture of a medicament for treating an inflammatory condition.

39. A method comprising,

increasing or stabilizing HMWK in an animal in need thereof by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

40. A method of treating an inflammatory condition in an animal in need thereof by increasing or stabilizing HMWK in the animal by administering to the animal a therapeutically effective amount of a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is at least 90% complementary to a kallikrein nucleic acid.

41. The method of claim 40, wherein the inflammatory condition is associated with low levels of HMWK.

42. The method of claim 40, wherein the inflammatory condition is associated with high levels of bradykinin.

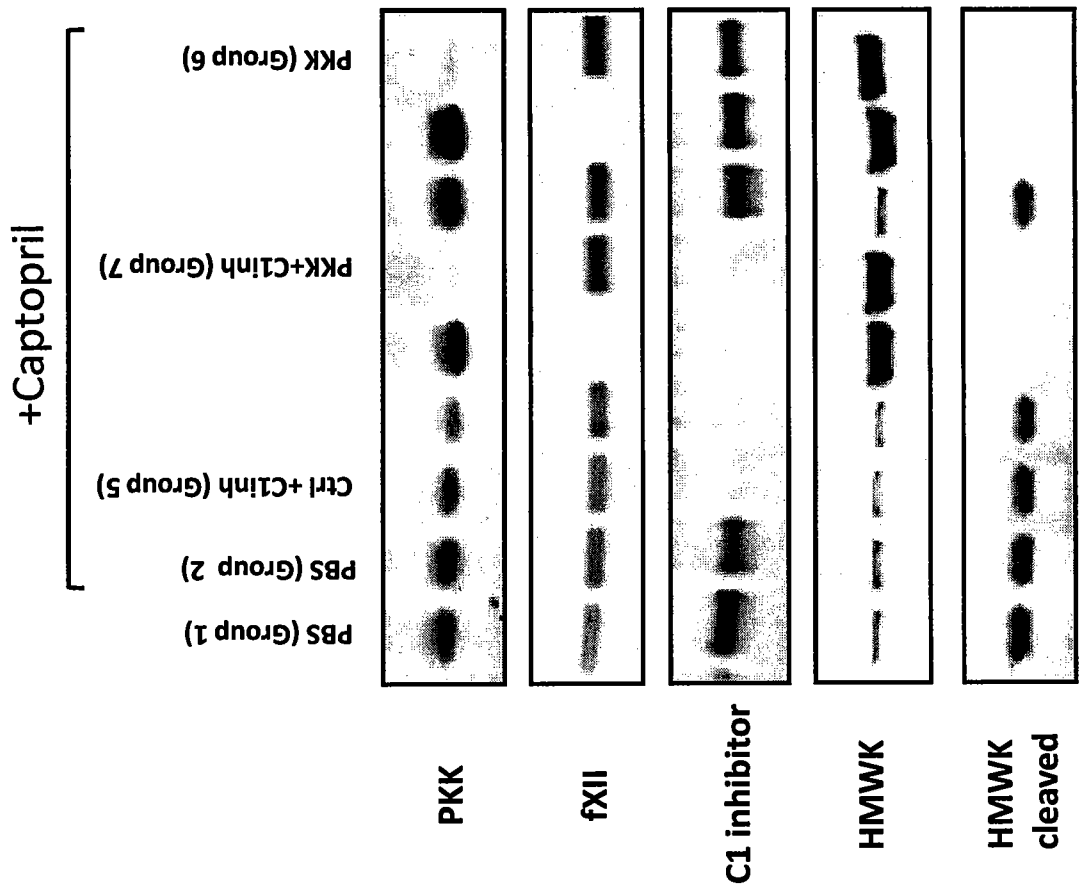

+Captopril
PBS (Group 1)
PBS (Group 2)
Ctrl +C1inh (Group 5)
PKK+C1inh (Group 7)
PKK (Group 6)
PKK
fXII
C1 inhibitor
HMWK
HMWK cleaved

Figure 1

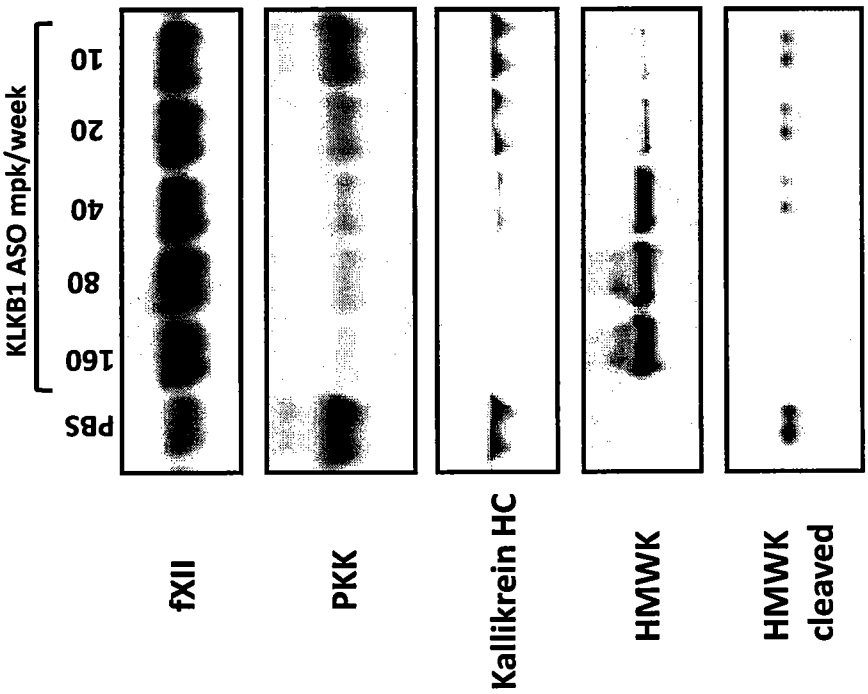

KLKB1 ASO mpk/week
10
20
40
80
160
PBS
fXII
PKK
Kallikrein HC
HMWK
HMWK cleaved

Figure 2