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(54) **Title**: MODULATION OF INFLAMMATORY RESPONSES BY FACTOR VII

(57) **Abstract**: Disclosed herein are antisense compounds and methods for modulating Factor VII and modulating an inflammatory disease, disorder or condition in an individual in need thereof. Inflammatory diseases in an individual such as arthritis and colitis can be treated, ameliorated or prevented with the administration of antisense compounds targeted to Factor VII. Provided herein are methods, compounds, and compositions for modulating levels of Factor VII mRNA and/or protein in an animal. Provided herein are methods, compounds, and compositions for modulating levels of Factor VII mRNA and/or protein in an animal in order to modulate an inflammatory response in the animal. Also provided herein are methods, compounds, and compositions for administering a therapeutically effective amount of a compound targeting Factor VII to an animal for ameliorating an inflammatory disease in an animal; treating an animal at risk for an inflammatory disease.

MODULATION OF INFLAMMATORY RESPONSES BY FACTOR VII

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 BIOL0156WOSEQ.TXT created June 13, 2012, which is 60 kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention provides methods, compounds, and compositions for modulating an inflammatory response by administering a Factor VII modulator to an animal. The present invention also provides methods, compounds, and compositions for modulating an inflammatory response by administering a Factor VII inhibitor to an animal.

BACKGROUND OF THE INVENTION

Factor VII

Factor VII (also known as preconvertin), an enzyme of the serine protease family, is part of the coagulation cascade to form blood clots. The coagulation cascade may be initiated through two branches, the tissue factor pathway (also "extrinsic pathway"), which is the primary pathway, and the contact activation pathway (also "intrinsic pathway").

The tissue factor pathway is initiated by the cell surface receptor tissue factor (TF, also referred to as factor III), which is expressed constitutively by extravascular cells (pericytes, cardiomyocytes, smooth muscle cells, and keratinocytes) and expressed by vascular monocytes and endothelial cells upon induction by inflammatory cytokines or endotoxin. (Drake *et al.*, *Am J Pathol* 1989, 134:1087-1097). TF is the high affinity cellular receptor for coagulation factor VIIa, a serine protease. In the absence of TF, VIIa has very low catalytic activity, and binding to TF is necessary to render VIIa functional through an allosteric mechanism. (Drake *et al.*, *Am J Pathol* 1989, 134:1087-1097). The TF-VIIa complex activates factor X to Xa. Xa in turn associates with its co-factor factor Va into a prothrombinase complex which in turn activates prothrombin, (also known as factor II or factor 2) to thrombin (also known as factor IIa, or factor

2a). Thrombin activates platelets, converts fibrinogen to fibrin and promotes fibrin cross-linking by activating factor XIII, thus forming a stable plug at sites where TF is exposed on extravascular cells. In addition, thrombin reinforces the coagulation cascade response by activating factors V and VIII.

The contact activation pathway is triggered by activation of factor XII to XIIa. Factor XIIa converts XI to XIa, and XIa converts IX to IXa. IXa associates with its cofactor VIIIa to convert X to Xa. The two pathways converge at this point as factor Xa associates factor Va to activate prothrombin (factor II) to thrombin (factor IIa).

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, IL-12, 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.

Treatment to decrease inflammation includes non-steroidal anti-inflammatory drugs (NSAIDS) as well as disease modifying drugs. Many of these drugs have unwanted side effects. For example, with NSAIDS, the most common side effects are nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, and drowsiness. NSAIDs may also cause fluid retention, leading to edema. The most serious side effects are kidney failure, liver failure, ulcers and prolonged bleeding after an injury or surgery.

Accordingly, there is a need to find alternative treatments for inflammation with more attractive clinical profiles. Little is known about the role of Factor VII in inflammation making it an attractive target for investigation. Antisense technology is emerging as an effective means for reducing the expression of certain gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of Factor VII.

SUMMARY OF THE INVENTION

Provided herein are methods, compounds, and compositions for modulating levels of Factor VII mRNA and/or protein in an animal. Provided herein are methods, compounds, and compositions for modulating levels of Factor VII mRNA and/or protein in an animal in order to modulate an inflammatory response in the animal. Also provided herein are methods, compounds, and compositions for administering a therapeutically effective amount of a compound targeting Factor VII to an animal for ameliorating an inflammatory disease in an animal; treating an animal at risk for an inflammatory disease; inhibiting Factor VII expression in an animal suffering from an inflammatory disease; and reducing the risk of inflammatory disease in an animal. In certain embodiments, the Factor VII modulator or compound targeting Facotr VII is a Factor VII specific inhibitor.

In certain embodiments, Factor VII specific inhibitors modulate (i.e., decrease) levels of Factor VII mRNA and/or protein. In certain embodiments, Factor VII specific inhibitors are nucleic acids, proteins, or small molecules.

In certain embodiments, an animal at risk for an inflammatory disease is treated by administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor VII nucleic acid as shown in any of SEQ ID NOs: 1-5.

In certain embodiments, an animal having an inflammatory disease is treated by administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor VII nucleic acid as shown in any of SEQ ID NOs: 1-5. In certain embodiments, an animal having an inflammatory disease is treated by administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to a target segment or target region of SEQ ID NOs: 1-5 as described herein. In certain embodiments the modified oligonucleotide has a nucleobase sequence comprising a contiguous nucleobase portion complementary to a target segment or target region of SEQ ID NOs: 1-5 as described herein.

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

Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions related to inflammation. In certain embodiments, such diseases, disorders, and conditions are inflammatory diseases, disorders or conditions.

In certain embodiments, methods of treatment include administering a Factor VII specific inhibitor to an individual in need thereof.

In certain embodiments, the inflammation is not sepsis related. In certain embodiments, the inflammation is not related to infection.

Also provided are compounds and compositions that include a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor VII nucleic acid as shown in SEQ ID NOs: 1-5. In certain embodiments the modified oligonucleotide has a nucleobase sequence comprising a contiguous nucleobase portion complementary to a target segment or target region of SEQ ID NOs: 1-5 as described herein. In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to a target segment or target region of SEQ ID NOs: 1-5 as described herein.

DETAILED DESCRIPTION OF THE INVENTION

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

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

Definitions

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

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

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

"2'-O-methoxyethyl nucleotide" means a nucleotide comprising a 2'-O-methoxyethyl modified sugar moiety.

"5-methylcytosine" means a cytosine modified with a methyl group attached to the 5' position. A 5-methylcytosine is a modified nucleobase.

"About" means within ±10% of a value. For example, if it is stated "the compounds inhibited Factor VII by at least about 70%", it is implied that the Factor VII levels are inhibited within a range of 63% to 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 Factor VII is an active pharmaceutical agent.

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

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

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

"Amelioration" refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. In certain embodiments, amelioration includes a delay or slowing in the progression of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art. For example, amelioration of arthritis in collagen-induced arthritic mice can be determined by clinically scoring the amount of arthritis in the mice as described by Marty et al. (J. Clin. Invest 107:631-640 (2001)).

"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.

"Antidote compound" refers to a compound capable decreasing the intensity or duration of any antisense activity.

"Antidote oligonucleotide" means an antidote compound comprising an oligonucleotide that is complementary to and capable of hybridizing with an antisense compound.

"Antidote protein" means an antidote compound comprising a peptide.

"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 is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

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

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

"Bicyclic sugar" means a furosyl ring modified by the bridging of two non-geminal ring atoms. A bicyclic sugar is a modified sugar.

"Bicyclic nucleic acid" or "BNA" refers to a nucleoside or nucleotide wherein the furanose portion of the nucleoside or nucleotide includes a bridge connecting two carbon atoms on the furanose ring, thereby forming a bicyclic ring system.

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

"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 concomitant, parallel or sequential administration.

"Coagulation factor" means any of factors I, II, III, IV, V, VII, VIII, IX, X, VII, VIII, or VIIII 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.

"Constrained ethyl" or "cEt" refers to a bicyclic nucleoside having a furanosyl sugar that comprises a methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') bridge between the 4' and the 2' carbon atoms.

"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.

"Disease modifying drug", "Disease modifying anti-inflammatory drug" or "DMARD" refers to any agent that modifies the symptoms and/or progression associated with an inflammatory disease, disorder or condition, including autoimmune diseases (e.g. arthritis, colitis or diabetes), trauma or surgery-related disorders, sepsis, allergic inflammation and asthma. DMARDs modify one or more of the symptoms and/or disease progression associated with these diseases, disorders or conditions.

"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.

"Factor VII", "FVII", "Factor 7" and "F7" is used interchangeably herein.

"Factor VII nucleic acid" or "Factor VII nucleic acid" means any nucleic acid encoding Factor VII. For example, in certain embodiments, a Factor VII nucleic acid includes a DNA sequence encoding Factor VII, an RNA sequence transcribed from DNA encoding Factor VII (including genomic DNA comprising introns and exons), and an mRNA sequence encoding Factor VII. "Factor VII mRNA" means an mRNA encoding a Factor VII protein.

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

"Factor VII specific inhibitor antidote" means a compound capable of decreasing the effect of a Factor VII specific inhibitor. In certain embodiments, a Factor VII specific inhibitor antidote is selected from a Factor VII peptide; a Factor VII antidote oligonucleotide, including a Factor VII antidote compound complementary to a Factor VII antisense compound; and any compound or protein that affects the intrinsic or extrinsic coagulation pathway.

"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 segment" and the external regions may be referred to as "wing segments."

"Gap-widened" means a chimeric antisense compound having a gap segment of 12 or more contiguous 2'-deoxynucleosides 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 an inflammatory disease, disorder or condition" means identifying an animal having been diagnosed with an inflammatory disease, disorder or condition or identifying an animal predisposed to develop an inflammatory disease, disorder or condition. Individuals predisposed to develop an inflammatory disease, disorder or condition, for example in individuals with a familial history of colitis or arthritis. 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 response" refers to any disease, disorder or condition related to inflammation in an animal. Examples of inflammatory responses include an immune response by the body of the animal to clear the injury or stimulus responsible for initiating the inflammatory response. Alternatively, an inflammatory response can be initiated in the body even when no known injury or stimulus is found such as in autoimmune diseases. Inflammation can be mediated by a Th1 or a Th2 response. Th1 and Th2 responses include production of selective cytokines and cellular migration or recruitment to the inflammatory site. Cell types that can migrate to an inflammatory site include, but are not limited to, eosinophils and macrophages. Th1 cytokines include, but are not limited to IL-1, IL-6, TNFα, INFγ and keratinocyte chemoattractanct (KC). Th2 cytokines include, but are not limited to, IL-4 and IL-5. A decrease in cytokine(s) level or cellular migration can be an indication of decreased inflammation.

Accordingly, cytokine level or cellular migration can be a marker for certain types of inflammation such as Th1 or Th2 mediated inflammation.

"Inflammatory disease", "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.

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

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

"Mismatch" or "non-complementary nucleobase" or "MM" 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.

"Modulating" refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating Factor VII mRNA can mean to increase or decrease the level of Factor VII mRNA and/or Factor VII protein in a cell, tissue, organ or organism. Modulating Factor VII mRNA and/or protein can lead to an increase or decrease in an inflammatory response in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, a Factor VII antisense oligonucleotide can be a modulator that increases or decreases the amount of Factor VII mRNA and/or Factor VII protein in a cell, tissue, organ or organism."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).

"NSAID" refers to a Non-Steroidal Anti-Inflammatory Drug. NSAIDs reduce inflammatory reactions in a subject but in general do not ameliorate or prevent a disease from occurring or progressing.

"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.

"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 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 is a modified internucleoside linkage.

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

"Prevent" refers to delaying or forestalling the onset, development or progression 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.

"Th1 related disease, disorder or condition" means an inflammatory disease, disorder or condition mediated by a Th1 immune response. Examples of Th1 diseases include, but is not limited to, allergic diseases (e.g., allergic rhinitis), autimmune diseases (e.g, multiple sclerosis, arthritis, scleroderma, psoriasis, celiac disease), cardiovascular diseases, colitis, diabetes (e.g., type 1 insulin-dependent diabetes mellitus), hypersensitivities (e.g., Type 4 hypersensitivity), infectious diseases (e.g., viral infection, mycobacterial infection) and posterior uveitis.

"Th2 related disease, disorder or condition" means an inflammatory disease, disorder or condition mediated by a Th2 immune response. Examples of Th2 diseases include, but is not limited to, allergic diseases (e.g, chronic rhinosinusitis), airway hyperresponsiveness, asthma, atopic dermatitis, colitis, endometriosis, infectious diseases (e.g., helminth infection), thyroid disease (e.g., Graves' disease), hypersensitivities (e.g, Types 1, 2 or 3 hypersensitivity) and pancreatitis.

"Th1" or "Th2" responses include production of selective cytokines and cellular migration or recruitment to an inflammatory site. Cell types that can migrate to an inflammatory site include, but are not limited to, eosinophils and macrophages. Accordingly, cytokine level or cellular migration can be a marker for certain types of inflammation such as Th1 or Th2 mediated inflammation. Th1 markers include, but are not limited to cytokines IL-1, IL-6, TNFα, INFγ and keratinocyte chemoattractanct (KC). Th2 markers include, but are not limited to, eosinophil infiltration, mucus production and cytokines IL-4 and IL-5. A decrease in cytokine(s) level or cellular migration can be an indication of decreased inflammation.

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

"Treat" refers to administering a pharmaceutical composition to an animal in order to effect an alteration or improvement of a disease, disorder, or condition in the animal. In certain embodiments, one or more pharmaceutical compositions can be administered to the animal.

"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-ribonucleotide) or a DNA nucleotide (i.e. β-D-deoxyribonucleotide).

Certain Embodiments

In certain embodiments, provided are Factor VII modulators. In certain embodiments, the Factor VII modulators can be Factor VII specific inhibitors, for use in treating, preventing, or ameliorating an inflammatory response, disease, disorder or condition. In certain embodiments, Factor VII specific inhibitors are nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of Factor VII mRNA and/or Factor VII protein. In certain embodiments, the Factor VII specific inhibitors are modified oligonucleotides. In certain embodiments, the Factor VII specific inhibitors decrease Factor VII expression.

In certain embodiments, provided are compound targeting Factor VII. In certain embodiments, the compounds targeting Factor VII can be Factor VII specific inhibitors, for use in treating, preventing, or ameliorating an inflammatory response, disease, disorder or condition. In certain embodiments, Factor VII specific inhibitors are nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of Factor VII mRNA and/or Factor VII protein. In certain embodiments, the Factor VII specific inhibitors are modified oligonucleotides. In certain embodiments, the Factor VII specific inhibitors decrease Factor VII expression.In certain embodiments, provided are compounds targeted to a Factor VII nucleic acid. In certain embodiments, the Factor VII nucleic acid is any of the human sequences set forth in GENBANK Accession No. NM_000131.3 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NM_019616.2 (incorporated herein as SEQ ID NO: 2) and nucleotides 1255000 to 1273000 of GENBANK Accession No. NT_027140.6 (incorporated herein as SEQ ID NO: 3). In certain embodiments, the Factor VII nucleic acid is any of the murine sequences set forth in GENBANK Accession NM_010172.3 (incorporated herein as SEQ ID NO: 4) and nucleotides 10024000 to 10037000 of GENBANK Accession No. NT_039455.6 (incorporated herein as SEQ ID NO: 5).

In certain embodiments, provided for use in the methods are compounds comprising a modified oligonucleotide. In certain embodiments, the compounds comprise a modified oligonucleotide consisting of 12 to 30 linked nucleosides.

In certain embodiments, the compounds for use in the methods 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 NOs: 1-5. In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NOs: 1-5.

In certain embodiments, the compounds for use in the methods comprises a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of a target region as set out below as nucleobase ranges on the target RNA sequence.

In certain embodiments, provided are compounds for use in the methods comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 or at least 20 contiguous nucleobases of a nucleobase sequence complementary to any of the sequences recited in SEQ ID NOs: 1-5.

In certain embodiments, the modified oligonucleotide for use in the methods consists of 12 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 linked nucleosides.

In certain embodiments, the compound for use in the methods consists of a single-stranded modified oligonucleotide.

In certain embodiments, the compound for use in the methods has at least one modified internucleoside linkage. In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, each modified internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, the compound for use in the methods has at least one nucleoside comprising a modified sugar. In certain embodiments, at least one modified sugar is a bicyclic sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl (2'MOE).

In certain embodiments, the compound for use in the methods has at least one nucleoside comprising a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide of the compound for use in the methods 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 certain embodiments, the modified oligonucleotide of the compound for use in the methods 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 certain embodiments, the modified oligonucleotide of the compound for use in the methods comprises: (i) a gap segment consisting of eight to sixteen linked deoxynucleosides; (ii) a 5' wing segment consisting of two to six linked nucleosides; (iii) a 3' wing segment consisting of two to six 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 certain embodiments, provided are methods, compounds, and compositions for modulating an inflammatory response by administering the compound to an animal, wherein the compound comprises a Factor VII modulator. Modulation of Factor VII can lead to an increase or decrease of Factor VII mRNA and protein expression in order to increase or decrease an inflammatory response as needed. In certain embodiments, Factor VII inhibition in an animal is reversed by administering a modulator targeting Factor VII. In certain embodiments of the invention, Factor VII is inhibited by the modulator. The Factor VII modulator can be a modified oligonucleotide targeting Factor VII.

In certain embodiments, provided are methods, compounds, and compositions for the treatment, prevention, or amelioration of inflammatory diseases, disorders and conditions associated with Factor VII in an animal in need thereof. In an embodiment, the method for

ameliorating an inflammatory disease in an animal comprises administering to the animal a compound targeting Factor VII.

In certain embodiments provided are methods, compounds and compositions for treating an animal at risk for an inflammatory disease, disorder or condition, comprising administering a therapeutically effective amount of a compound targeting Factor VII to the animal at risk.

In certain embodiments, provided are methods, compounds and compositions for inhibiting Factor VII expression in an animal suffering from an inflammatory disease, disorder or condition, comprising administering a compound targeting Factor VII to the animal.

In certain embodiments, provided are methods, compounds and compositions for reducing the risk of inflammatory disease, disorder or condition, in an animal comprising administering a compound targeting Factor VII to the animal.

In certain embodiments, provided is a method comprising selecting an animal suffering from, or at risk of, an inflammatory disease, disorder or condition and administering a compound targeting Factor VII to the animal.

In certain embodiments, provided are methods, compounds and compositions for treating an animal at risk for an inflammatory disease, disorder or condition or an animal having an inflammatory disease, disorder or condition comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor VII nucleic acid as shown in SEQ ID NOs: 1-5.

In certain embodiments, provided are methods, compounds and compositions for treating an animal at having an inflammatory disease, disorder or condition or an animal having an inflammatory disease, disorder or condition comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor VII nucleic acid as shown in SEQ ID NOs: 1-5.

In certain embodiments, administration of a Factor VII modulator to an animal does not cause injurious bleeding in the animal or exacerbate a bleeding condition.

In certain embodiments, the animal is pre-treated with one or more Factor VII modulators.

In certain embodiments, the animal is a human.

In certain embodiments, provided are methods, compounds, and compositions for modulating a symptom or therapeutic endpoint of an inflammatory disease or condition. The symptom or therapeutic endpoint can be selected from one or more of colon length, diarrhea, colon vascular permeability or mucus production. In certain embodiments, diarrhea, mucus production and colon vascular permeability is decreased. In certain embodiments, shrinkage of colon length is ameliorated or prevented. In certain embodiments, the colon length is increased.

In certain embodiments, provided are methods, compounds and compositions for modulating a marker of an inflammatory response. The marker can be selected from one or more of colon length, diarrhea, colon vascular permeability, mucus production, eosinophil recruitment, neutrophil recruitment, lymphocyte recruitment, kinin level, kallikrein activity or cytokine levels.

Examples of cytokines include INF-γ, IL-1β, IL-6, TNF-α and KC.

In certain embodiments, the compounds of the invention treats, prevents or ameliorates an inflammatory response, disease, disorder or condition in an animal. In certain embodiments, the inflammatory response, disease, disorder or condition is associated with Factor VII. In certain embodiments the inflammatory response, disease, disorder, or condition may include, but is not limited to, or may be due to or associated with, arthritis, colitis, embolism, fibrosis, allergic inflammation, asthma, cardiovascular disease, diabetes, sepsis, antiphospholipid syndrome, graft-related diseases and autoimmune diseases, or any combination thereof.

Examples of fibrosis include pulmonary fibrosis, cirrhosis, endomyocardial fibrosis, dediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, nephrogenic fibrosis, Crohn's Disease, Keloids, scleroderma, arthrofibrosis and the like.

Examples of embolism include pulmonary embolism, arterial embolism, venous embolism and the like.

Examples of arthritis include, but are not limited to, rheumatoid arthritis, juvenile rheumatoid arthritis, arthritis uratica, gout, chronic polyarthritis, periarthritis humeroscapularis, cervical arthritis, lumbosacral arthritis, osteoarthritis, psoriatic arthritis, enteropathic arthritis and ankylosing spondylitis.

Examples of colitis include, but are not limited to, ulcerative colitis, Inflammatory Bowel Disease (IBD) and Crohn's Disease.

Examples of graft-related disorders include, but are not limited to, graft versus host disease (GVHD), disorders associated with graft transplantation rejection, chronic rejection, and tissue or cell allografts or xenografts.

Examples of autoimmune diseases include, but are not limited to, lupus (e.g., lupus erythematosus, lupus nephritis), Hashimoto's thyroiditis, primary myxedema, Graves' disease, pernicious anemia, autoimmune atrophic gastritis, Addison's disease, diabetes (e.g. insulin dependent diabetes mellitus, type I diabetes mellitus, type II diabetes mellitus), good pasture's syndrome, myasthenia gravis, pemphigus, Crohn's disease, sympathetic ophthalmia, autoimmune uveitis, multiple sclerosis, autoimmune hemolytic anemia, idiopathic thrombocytopenia, primary biliary cirrhosis, chronic action hepatitis, ulcerative colitis, Sjogren's syndrome, rheumatic diseases (e.g., rheumatoid arthritis), polymyositis, scleroderma, psoriasis, and mixed connective tissue disease.

In certain embodiments, the inflammatory disease, disorder or condition is a fibrin related inflammatory disease, disorder or condition.

In certain embodiments, the inflammatory disease, disorder or condition is Th1 mediated. In certain embodiments, a marker for the Th1 mediated inflammatory disease, disorder or condition is decreased. Markers for Th1 include, but are not limited to cytokines such as IL-1, IL-6, INF-γ, TNF-α or KC. In certain embodiments, the compounds of the invention prevent or ameliorate a Th1 mediated disease. Th1 mediated diseases include, but is not limited to, allergic diseases (e.g., allergic rhinitis), autimmune diseases (e.g, multiple sclerosis, arthritis, scleroderma, psoriasis, celiac disease), cardiovascular diseases, colitis, diabetes (e.g., type 1 insulin-dependent diabetes mellitus), hypersensitivities (e.g., Type 4 hypersensitivity), infectious diseases (e.g., viral infection, mycobacterial infection) and posterior uveitis.

In certain embodiments, the inflammatory disease, disorder or condition is Th2 mediated. In certain embodiments, a marker for the Th2 mediated inflammatory disease, disorder or condition is decreased. Markers for Th2 include, but are not limited to, eosinophil infiltration to the site of inflammation, mucus production and cytokines such as IL-4, IL-5. In certain embodiments, the compounds of the invention prevent or ameliorate a Th2 mediated disease. Th2 mediated diseases include, but is not limited to, allergic diseases (e.g, chronic rhinosinusitis), airway hyperresponsiveness, asthma, atopic dermatitis, colitis, endometriosis,

infectious diseases (e.g., helminth infection), thyroid disease (e.g., Graves' disease), hypersensitivities (e.g, Types 1, 2 or 3 hypersensitivity) and pancreatitis.

In certain embodiments, the compounds and compositions are administered to an animal to treat, prevent or ameliorate an inflammatory disease. In certain embodiments, administration to an animal is by a parenteral route. In certain embodiments, the parenteral administration is any of subcutaneous or intravenous administration.

In certain embodiments, the compound is co-administered with one or more second agent(s). In certain embodiments the second agent is a NSAID or a disease modifying drug.

NSAIDS include, but are not limited to, acetyl salicylic acid, choline magnesium salicylate, diflunisal, magnesium salicylate, salsalate, sodium salicylate, diclofenac, etodolac, fenoprofen, flurbiprofen, indomethacin, ketoprofen, ketorolac, meclofenamate, naproxen, nabumetone, phenylbutazone, piroxicam, sulindac, tolmetin, acetaminophen, ibuprofen, Cox-2 inhibitors, meloxicam and tramadol. The compound of the invention and one or more NSAIDS can be administered concomitantly or sequentially.

Examples of disease modifying drugs include, but are not limited to, methotrexate, abatacept, infliximab, cyclophosphamide, azathioprine, corticosteroids, cyclosporin A, aminosalicylates, sulfasalazine, hydroxychloroquine, leflunomide, etanercept, efalizumab, 6-mercapto-purine (6-MP), and tumor necrosis factor-alpha (TNFalpha) or other cytokine blockers or antagonists. The compound of the invention and one or more disease modifying drug can be administered concomitantly or sequentially.

In certain embodiments, a compound or oligonucleotide is in salt form.

In certain embodiments, the compounds or compositions are formulated with a pharmaceutically acceptable carrier or diluent.

In certain embodiments, provided are methods and compounds useful for the treatment, prevention, or amelioration of an inflammatory response or inflammatory disease, disorder, or condition. Factor VII has a sequence as shown in any of SEQ ID NOs: 1-5. In certain embodiments, a modified oligonucleotide is used for treating an inflammatory response or inflammatory disease, disorder, or condition.

In certain embodiments, provided are methods and compounds useful in the manufacture of a medicament for the treatment, prevention, or amelioration of an inflammatory response or inflammatory disease, disorder, or condition Factor VII has a sequence as shown in any of SEQ

ID NOs: 1-5. In certain embodiments, a modified oligonucleotide is used in the manufacture of a medicament for treating an inflammatory response or inflammatory disease, disorder, or condition. In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising a portion of nucleobases complementary to any of SEQ ID NOs: 1-5. In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to any of SEQ ID NOs: 1-5.

In certain embodiments, provided is the use of a Factor VII modulator as described herein in the manufacture of a medicament for treating, ameliorating, or preventing inflammatory diseases, disorders, and conditions associated with Factor VII.

In certain embodiments, provided is a Factor VII modulator as described herein for use in treating, preventing, or ameliorating an inflammatory response or inflammatory disease, disorder, or condition as described herein. The Factor VII modulator can be used in combination therapy with one or more additional agent or therapy as described herein. Agents or therapies can be administered concomitantly or sequentially to an animal.

In certain embodiments, provided is the use of a Factor VII modulator as described herein in the manufacture of a medicament for treating, preventing, or ameliorating an inflammatory disease, disorder or condition as described herein. In certain embodiments, the Factor VII modulator is a Factor VII specific inhibitor, for use in treating, preventing, or ameliorating an inflammatory response, disease, disorder or condition. In certain embodiments, the Factor VII specific inhibitor is a nucleic acid (including antisense compound), peptide, antibody, small molecule, or other agent capable of inhibiting the expression of Factor VII mRNA and/or Factor VII protein. In certain embodiments, the Factor VII specific inhibitor is a modified oligonucleotide. In certain embodiments, the Factor VII specific inhibitor decreases Factor VII expression.The Factor VII modulator can be used in combination therapy with one or more additional agent or therapy as described herein. Agents or therapies can be administered concomitantly or sequentially to an animal.

In certain embodiments, provided is a kit for treating, preventing, or ameliorating an inflammatory response, disease, disorder or condition as described herein wherein the kit comprises: (i) a Factor VII specific inhibitor as described herein; and optionally (ii) an additional agent or therapy as described herein.

A kit of the present invention may further include instructions for using the kit to treat,

prevent, or ameliorate an inflammatory disease, disorder or condition as described herein by combination therapy as described herein.

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 can be "antisense" to a target nucleic acid, meaning that 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 Factor VII nucleic acid is 10 to 30 nucleotides in length. In other words, antisense compounds are from 10 to 30 linked nucleobases. In other embodiments, the antisense compound comprises a modified oligonucleotide consisting of 8 to 80, 10 to 80, 12 to 50, 15 to 30, 18 to 24, 19 to 22, or 20 linked nucleobases. In certain such embodiments, the antisense compound comprises a modified oligonucleotide consisting of 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 nucleobases in length, or a range defined by any two of the above values. In some embodiments, the antisense compound is an antisense oligonucleotide.

In certain embodiments, the antisense compound comprises a shortened or truncated modified oligonucleotide. The shortened or truncated modified oligonucleotide can have a single nucleoside deleted from the 5' end (5' truncation), the central portion or alternatively from the 3' end (3' truncation). A shortened or truncated oligonucleotide can have two or more nucleosides deleted from the 5' end, two or more nucleosides deleted from the central portion or alternatively can have two or more nucleosides deleted from the 3' end. Alternatively, the deleted nucleosides can be dispersed throughout the modified oligonucleotide, for example, in an

antisense compound having one or more nucleoside deleted from the 5' end, one or more nucleoside deleted from the central portion and/or one or more nucleoside deleted from the 3' end.

In certain embodiments, the antisense compound comprises a lengthened or long modified oligonucleotide. When a single additional nucleoside is present in a lengthened oligonucleotide, the additional nucleoside can be located at the 5' end, 3' end or central portion of the oligonucleotide. When two or more additional nucleosides are present, the added nucleosides can be adjacent to each other, for example, in an oligonucleotide having two nucleosides added to the 5' end (5' addition), to the 3' end (3' addition) or the central portion, of the oligonucleotide. Alternatively, the added nucleoside can be dispersed throughout the antisense compound, for example, in an oligonucleotide having one or more nucleoside added to the 5' end, one or more nucleoside added to the 3' end, and/or one or more nucleoside added to the central portion.

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 Factor VII nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced the 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 can 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 can in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides can include 2'-MOE, and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides can include those having a 4'-(CH2)n-O-2' bridge, where n=1 or n=2). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap segment is positioned immediately adjacent each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing segment and gap segment, or the gap segment

and the 3' wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotides. Thus, gapmers 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, 6-8-6, 5-8-5, 1-8-1, 2-6-2, 6-8-6, 5-8-5, 1-8-1, 2-6-2, 2-13-2, 1-8-2, 2-8-3, 3-10-2, 1-18-2, or 2-18-2.

In certain embodiments, the antisense compound as 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 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, or 5-13.

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

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

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

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

In certain embodiments, a gap-widened antisense oligonucleotide targeted to a Factor VII nucleic acid has a gap segment of fourteen 2'-deoxyribonucleosides 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 Factor VII nucleic acid has a gap segment of thirteen 2'-deoxyribonucleosides 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 Factor VII include, without limitation, the following: GENBANK Accession No. NM_000131.3 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NM_019616.2 (incorporated herein as SEQ ID NO: 2), nucleotides 1255000 to 1273000 of GENBANK Accession No. NT_027140.6 (incorporated herein as SEQ ID NO: 3), GENBANK Accession NM_010172.3 (incorporated herein as SEQ ID NO: 4) and nucleotides 10024000 to 10037000 of GENBANK Accession No. NT_039455.6 (incorporated herein as SEQ ID NO: 5).

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 Factor VII 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 target region.

In certain embodiments, a "target segment" is a smaller, sub-portion of a target region within a nucleic acid. For example, a target segment can be the sequence of nucleotides of a target nucleic acid to which one or more 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.

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 Factor VII mRNA levels are indicative of inhibition of Factor VII expression. Reductions in levels of a Factor VII protein are also indicative of inhibition of target mRNA levels. Further, phenotypic changes are indicative of inhibition of Factor VII expression. For example, a decrease in colon shrinkage can be indicative of inhibition of Factor VII expression. In another example, a decrease in diarrhea can be indicative of inhibition of Factor VII expression. In another example, a decrease in colon vascular permeability can be indicative of

inhibition of Factor VII expression. In another example, reduced formation of inflammation (e.g., in fibrosis, embolism, asthma or colitis formation) can be indicative of inhibition of Factor VII expression. Alternatively, increased time for inflammation formation (e.g, in fibrosis, embolism, asthma or colitis formation) can be indicative of inhibition of Factor VII expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a Factor VII 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 (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a Factor VII nucleic acid.

Complementarity

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

Non-complementary nucleobases between an antisense compound and a Factor VII 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 Factor VII 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 Factor VII 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, antisense compound may be fully complementary to a Factor VII 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 Factor VII 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 Factor VII 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.

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 Factor VII 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' (also referred to as constrained ethyl or cEt) 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

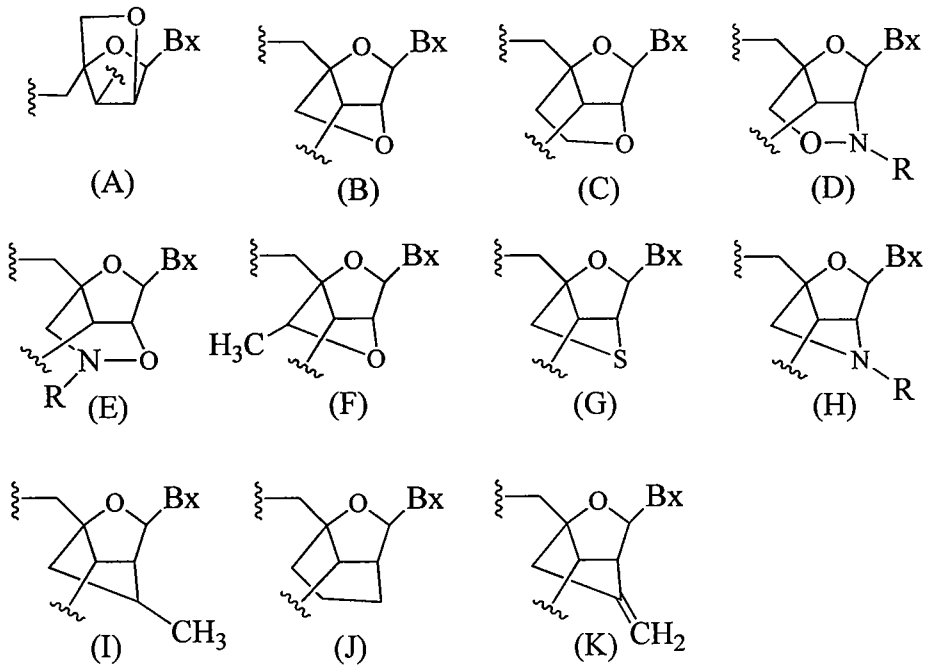
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted

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-, -$C(R_aR_b)$-N(R)-O- or –$C(R_aR_b)$-O-N(R)-. In certain embodiments, the bridge is 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R)-2' and 4'-CH_2-N(R)-O-2'- wherein each R is, independently, H, a protecting group or C_1-C_{12} alkyl.

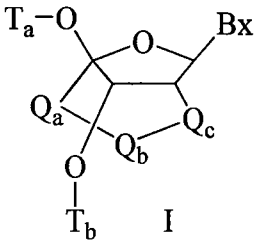
In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH_2-O-2') BNA's have been incorporated into antisense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

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-methyleneoxy (4'-CH_2-O-2') BNA , (B) β-D-methyleneoxy (4'-CH_2-O-2') BNA , (C) ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA , (D) aminooxy (4'-CH_2-O-N(R)-2') BNA, (E) oxyamino (4'-CH_2-N(R)-O-2') BNA, 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, (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA and (K) vinyl BNA as depicted below.



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

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



I

wherein:

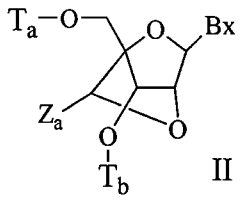
Bx is a heterocyclic base moiety;

$-Q_a-Q_b-Q_c-$ 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:



II

wherein:

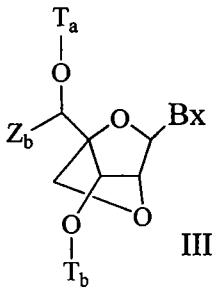
Bx is a heterocyclic base moiety;

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

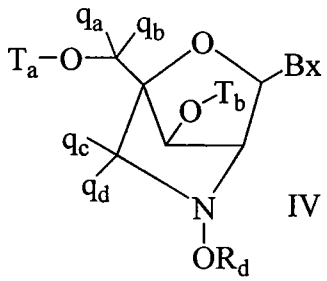
wherein:

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:

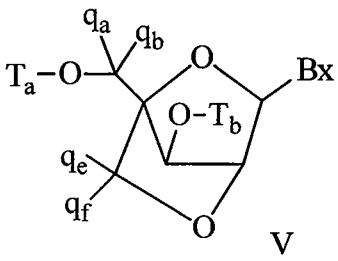
Bx is a heterocyclic base moiety;

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 $N(H)C(=S)NJ_jJ_k$;

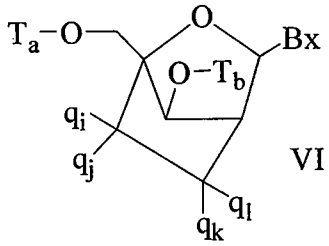
or q_e and q_f together are $=C(q_g)(q_h)$;

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

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).

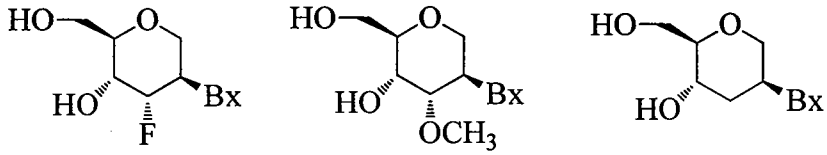
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

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.

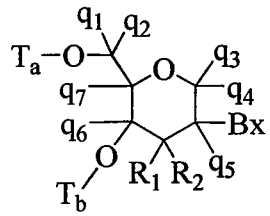
As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and 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) or fluoro HNA (F-HNA) having a tetrahydropyran ring system as illustrated below:



In certain embodiments, sugar surrogates are selected having Formula VII:



VII

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

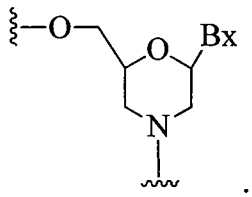
Bx is a heterocyclic base moiety;

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 methoxyethoxy and R_2 is H.

In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example nucleosides comprising morpholino sugar moieties and their use in oligomeric compounds has been reported (see for example: Braasch *et al.*, *Biochemistry*, 2002, *41*, 4503-4510; and U.S. Patents 5,698,685; 5,166,315; 5,185,444; and 5,034,506). As used here, the term "morpholino" means a sugar surrogate having the following formula:

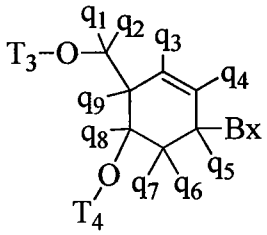


In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as "modifed morpholinos."

Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and 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 bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH_2-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.,* Srivastava *et al., J. Am. Chem. Soc.* 2007, *129(26)*, 8362-8379).

In certain embodiments, antisense compounds comprise one or more modified cyclohexenyl nucleosides, which is a nucleoside having a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Modified cyclohexenyl nucleosides include, but are not limited to those described in the art (see for example commonly owned, published PCT Application WO 2010/036696, published on April 10, 2010, Robeyns *et al., J. Am. Chem. Soc.,* 2008, *130(6)*, 1979-1984; Horváth *et al., Tetrahedron Letters,* 2007, *48*, 3621-3623; Nauwelaerts *et al., J. Am. Chem. Soc.*, 2007, *129(30)*, 9340-9348; Gu *et al.,*, *Nucleosides, Nucleotides & Nucleic Acids*, 2005, *24(5-7)*, 993-998; Nauwelaerts *et al., Nucleic*

Acids Research, 2005, *33(8)*, 2452-2463; Robeyns et al., *Acta Crystallographica, Section F: Structural Biology and Crystallization Communications,* 2005, *F61(6)*, 585-586; Gu *et al., Tetrahedron*, 2004, *60(9)*, 2111-2123; Gu *et al., Oligonucleotides*, 2003, *13(6)*, 479-489; Wang *et al., J. Org. Chem.,* 2003, *68*, 4499-4505; Verbeure *et al., Nucleic Acids Research*, 2001, *29(24)*, 4941-4947; Wang *et al., J. Org. Chem.,* 2001, *66*, 8478-82; Wang *et al., Nucleosides, Nucleotides & Nucleic Acids*, 2001, *20(4-7)*, 785-788; Wang *et al., J. Am. Chem.,* 2000, *122*, 8595-8602; Published PCT application, WO 06/047842; and Published PCT Application WO 01/049687; the text of each is incorporated by reference herein, in their entirety). Certain modified cyclohexenyl nucleosides have Formula X.



X

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

Bx is a heterocyclic base moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the cyclohexenyl nucleoside analog to an antisense compound or one of T_3 and T_4 is an internucleoside linking group linking the tetrahydropyran nucleoside analog to an antisense compound and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group, or a 5'-or 3'-terminal group; and

q_1, q_2, q_3, q_4, q_5, q_6, q_7, q_8 and q_9 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, substituted C_2-C_6 alkynyl or other sugar substituent group.

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 of the sugar ring.

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. Some representative U.S. patents that teach the preparation of such modified sugars include without limitation, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,670,633; 5,700,920; 5,792,847 and 6,600,032 and International Application PCT/US2005/019219, filed June 2, 2005 and published as WO 2005/121371 on December 22, 2005, and each of which is herein incorporated by reference in its entirety.

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.

Modified Nucleobases

Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications may impart nuclease stability, binding affinity or some other beneficial biological property to antisense compounds. Modified nucleobases include synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of an antisense compound for a target nucleic acid. For example, 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278).

Additional modified nucleobases include 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Nucleobases that are particularly useful for increasing the binding affinity of antisense compounds include 5-substituted pyrimidines, 6-azapyrimidines and

N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

In certain embodiments, antisense compounds targeted to a Factor VII nucleic acid comprise one or more modified nucleobases. In certain embodiments, gap-widened antisense oligonucleotides targeted to a Factor VII nucleic acid comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Compositions and Methods for Formulating Pharmaceutical Compositions

Antisense oligonucleotides can be admixed with pharmaceutically acceptable active or inert substance 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 Factor VII nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier.

In certain embodiments, the "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and can be selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

Pharmaceutically acceptable organic or inorganic excipients, which do not deleteriously react with nucleic acids, suitable for parenteral or non-parenteral administration can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

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 Factor VII 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.

In certain embodiments, one or more modified oligonucleotides of the present invention can be formulated as a prodrug. A prodrug can be produced by modifying a pharmaceutically active compound such that the active compound will be regenerated upon in vivo administration. For example, 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.

In certain embodiments, a pharmaceutical composition comprises a sterile lyophilized modified oligonucleotide that is reconstituted with a suitable diluent, e.g., sterile water for injection or sterile saline for injection. The reconstituted product is administered as a subcutaneous injection or as an intravenous infusion after dilution into saline. The lyophilized drug product consists of a modified oligonucleotide which has been prepared in water for injection, or in saline for injection, adjusted to pH 7.0-9.0 with acid or base during preparation,

and then lyophilized. The lyophilized modified oligonucleotide may be 25-800 mg, or any dose between 25-800 mg as described above, of a modified oligonucleotide. The lyophilized drug product may be packaged in a 2 mL Type I, clear glass vial (ammonium sulfate-treated), stoppered with a bromobutyl rubber closure and sealed with an aluminum flip-off overseal.

In certain embodiments, the compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. Such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the oligonucleotide(s) of the formulation.

In certain embodiments, pharmaceutical compositions of the present invention comprise one or more modified oligonucleotides and one or more excipients. In certain such embodiments, excipients are selected from water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and polyvinylpyrrolidone.

In certain embodiments, a pharmaceutical composition of the present invention is prepared using known techniques, including, but not limited to mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tabletting processes.

In certain embodiments, the compounds of the invention targeted to a Factor VII 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, but is not limited to, water, oils, alcohols, or 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 compound targeted to a Factor VII nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the compound is an antisense oligonucleotide.

In certain embodiments, a pharmaceutical composition of the present invention is a liquid (e.g., a suspension, elixir and/or solution). In certain of such embodiments, a liquid pharmaceutical composition is prepared using ingredients known in the art, including, but not limited to, water, buffered saline, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents.

In certain embodiments, a pharmaceutical composition of the present invention is a solid (e.g., a powder, tablet, and/or capsule). In certain of such embodiments, a solid pharmaceutical composition comprising one or more oligonucleotides is prepared using ingredients known in the art, including, but not limited to, starches, sugars, diluents, granulating agents, lubricants, binders, and disintegrating agents.

In certain embodiments, a pharmaceutical composition of the present invention is formulated as a depot preparation. Certain such depot preparations are typically longer acting than non-depot preparations. In certain embodiments, such preparations are administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. In certain embodiments, depot preparations are prepared using suitable polymeric or hydrophobic materials (for example an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

In certain embodiments, a pharmaceutical composition of the present invention comprises a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical compositions including those comprising hydrophobic compounds. In certain embodiments, certain organic solvents such as dimethylsulfoxide are used.

In certain embodiments, a pharmaceutical composition of the present invention comprises one or more tissue-specific delivery molecules designed to deliver the one or more pharmaceutical agents of the present invention to specific tissues or cell types. For example, in certain embodiments, pharmaceutical compositions include liposomes coated with a tissue-specific antibody.

In certain embodiments, a pharmaceutical composition of the present invention comprises a co-solvent system. Certain of such co-solvent systems comprise, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. In certain embodiments, such co-solvent systems are used for hydrophobic compounds. A non-limiting example of such a co-solvent system is the VPD co-solvent system, which is a solution of absolute ethanol comprising 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80™ and 65% w/v polyethylene glycol 300. The proportions of such co-solvent systems may be varied considerably without significantly altering their solubility and toxicity characteristics. Furthermore, the identity of co-solvent components may be varied: for example, other surfactants may be used instead of Polysorbate 80™; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

In certain embodiments, a pharmaceutical composition of the present invention comprises a sustained-release system. A non-limiting example of such a sustained-release system is a semi-permeable matrix of solid hydrophobic polymers. In certain embodiments, sustained-release systems may, depending on their chemical nature, release pharmaceutical agents over a period of hours, days, weeks or months.

In certain embodiments, a pharmaceutical composition of the present invention is prepared for oral administration. In certain of such embodiments, a pharmaceutical composition is formulated by combining one or more compounds comprising a modified oligonucleotide with one or more pharmaceutically acceptable carriers. Certain of such carriers enable pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject. In certain embodiments, pharmaceutical compositions for oral use are obtained by mixing oligonucleotide and one or more solid excipient. Suitable excipients include, but are not limited to, fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). In certain embodiments, such a mixture is optionally ground and auxiliaries are optionally added. In certain embodiments, pharmaceutical compositions are formed to obtain

tablets or dragee cores. In certain embodiments, disintegrating agents (e.g., cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate) are added.

In certain embodiments, dragee cores are provided with coatings. In certain such embodiments, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to tablets or dragee coatings.

In certain embodiments, pharmaceutical compositions for oral administration are push-fit capsules made of gelatin. Certain of such push-fit capsules comprise one or more pharmaceutical agents of the present invention in admixture with one or more filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In certain embodiments, pharmaceutical compositions for oral administration are soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. In certain soft capsules, one or more pharmaceutical agents of the present invention are be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

In certain embodiments, pharmaceutical compositions are prepared for buccal administration. Certain of such pharmaceutical compositions are tablets or lozenges formulated in conventional manner.

In certain embodiments, a pharmaceutical composition is prepared for administration by injection (e.g., intravenous, subcutaneous, intramuscular, etc.). In certain of such embodiments, a pharmaceutical composition comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer (e.g., PBS). In certain embodiments, other ingredients are included (e.g., ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Certain pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents

and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, such suspensions may also contain suitable stabilizers or agents that increase the solubility of the pharmaceutical agents to allow for the preparation of highly concentrated solutions.

In certain embodiments, a pharmaceutical composition is prepared for transmucosal administration. In certain of such embodiments penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

In certain embodiments, a pharmaceutical composition is prepared for administration by inhalation. Certain of such pharmaceutical compositions for inhalation are prepared in the form of an aerosol spray in a pressurized pack or a nebulizer. Certain of such pharmaceutical compositions comprise a propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In certain embodiments using a pressurized aerosol, the dosage unit may be determined with a valve that delivers a metered amount. In certain embodiments, capsules and cartridges for use in an inhaler or insufflator may be formulated. Certain of such formulations comprise a powder mixture of a pharmaceutical agent of the invention and a suitable powder base such as lactose or starch.

In certain embodiments, a pharmaceutical composition is prepared for rectal administration, such as a suppositories or retention enema. Certain of such pharmaceutical compositions comprise known ingredients, such as cocoa butter and/or other glycerides.

In certain embodiments, a pharmaceutical composition is prepared for topical administration. Certain of such pharmaceutical compositions comprise bland moisturizing bases, such as ointments or creams. Exemplary suitable ointment bases include, but are not limited to, petrolatum, petrolatum plus volatile silicones, and lanolin and water in oil emulsions. Exemplary suitable cream bases include, but are not limited to, cold cream and hydrophilic ointment.

In certain embodiments, a pharmaceutical composition of the present invention comprises a modified oligonucleotide in a therapeutically effective amount. In certain embodiments, the therapeutically effective amount is sufficient to prevent, alleviate or ameliorate symptoms of a disease or to prolong the survival of the subject being treated.

Dosing

In certain embodiments, pharmaceutical compositions are administered according to a dosing regimen (e.g., dose, dose frequency, and duration) wherein the dosing regimen can be selected to achieve a desired effect. The desired effect can be, for example, reduction of Factor VII or the prevention, reduction, amelioration or slowing the progression of a disease or condition associated with Factor VII.

In certain embodiments, the variables of the dosing regimen are adjusted to result in a desired concentration of pharmaceutical composition in a subject. "Concentration of pharmaceutical composition" as used with regard to dose regimen can refer to the compound, oligonucleotide, or active ingredient of the pharmaceutical composition. For example, in certain embodiments, dose and dose frequency are adjusted to provide a tissue concentration or plasma concentration of a pharmaceutical composition at an amount sufficient to achieve a desired effect.

Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Dosing is also dependent on drug potency and metabolism. In certain embodiments, dosage is from 0.01μg to 100mg per kg of body weight, or within a range of 0.001mg to 1000mg dosing, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01μg to 100mg per kg of body weight, once or more daily, to once every 20 years or ranging from 0.001mg to 1000mg dosing.

In certain embodiments, a pharmaceutical composition of the present invention is administered in the form of a dosage unit (e.g., tablet, capsule, bolus, etc.). In certain embodiments, such pharmaceutical compositions comprise a modified oligonucleotide in a dose selected from 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 105 mg, 110 mg, 115 mg, 120 mg, 125 mg, 130 mg, 135 mg, 140 mg, 145 mg, 150 mg, 155 mg, 160 mg, 165 mg, 170 mg, 175 mg, 180 mg, 185 mg, 190 mg, 195 mg, 200 mg, 205 mg, 210 mg, 215 mg, 220 mg, 225 mg, 230 mg, 235 mg, 240 mg, 245 mg, 250 mg, 255 mg, 260 mg, 265 mg, 270 mg, 270 mg, 280 mg, 285 mg, 290 mg, 295 mg, 300

mg, 305 mg, 310 mg, 315 mg, 320 mg, 325 mg, 330 mg, 335 mg, 340 mg, 345 mg, 350 mg, 355 mg, 360 mg, 365 mg, 370 mg, 375 mg, 380 mg, 385 mg, 390 mg, 395 mg, 400 mg, 405 mg, 410 mg, 415 mg, 420 mg, 425 mg, 430 mg, 435 mg, 440 mg, 445 mg, 450 mg, 455 mg, 460 mg, 465 mg, 470 mg, 475 mg, 480 mg, 485 mg, 490 mg, 495 mg, 500 mg, 505 mg, 510 mg, 515 mg, 520 mg, 525 mg, 530 mg, 535 mg, 540 mg, 545 mg, 550 mg, 555 mg, 560 mg, 565 mg, 570 mg, 575 mg, 580 mg, 585 mg, 590 mg, 595 mg, 600 mg, 605 mg, 610 mg, 615 mg, 620 mg, 625 mg, 630 mg, 635 mg, 640 mg, 645 mg, 650 mg, 655 mg, 660 mg, 665 mg, 670 mg, 675 mg, 680 mg, 685 mg, 690 mg, 695 mg, 700 mg, 705 mg, 710 mg, 715 mg, 720 mg, 725 mg, 730 mg, 735 mg, 740 mg, 745 mg, 750 mg, 755 mg, 760 mg, 765 mg, 770 mg, 775 mg, 780 mg, 785 mg, 790 mg, 795 mg, and 800 mg. In certain such embodiments, a pharmaceutical composition of the present invention comprises a dose of modified oligonucleotide selected from 25 mg, 50 mg, 75 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 500 mg, 600 mg, 700 mg, and 800 mg.

Administration

The compounds or pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be oral, inhaled or parenteral.

In certain embodiments, the compounds and compositions as described herein are administered parenterally. Parenteral administration includes intravenous, intra-arterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

In certain embodiments, parenteral administration is by infusion. Infusion can be chronic or continuous or short or intermittent. In certain embodiments, infused pharmaceutical agents are delivered with a pump.

In certain embodiments, parenteral administration is by injection. The injection can be delivered with a syringe or a pump. In certain embodiments, the injection is a bolus injection. In certain embodiments, the injection is administered directly to a tissue or organ.

In certain embodiments, formulations for parenteral, intrathecal or intraventricular administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

In certain embodiments, formulations for oral administration of the compounds or compositions can include, but is not limited to, pharmaceutical carriers, excipients, powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In certain embodiments, oral formulations are those in which compounds provided herein are administered in conjunction with one or more penetration enhancers, surfactants and chelators.

In certain embodiments, administration includes pulmonary administration. In certain embodiments, pulmonary administration comprises delivery of aerosolized oligonucleotide to the lung of a subject by inhalation. Following inhalation by a subject of aerosolized oligonucleotide, oligonucleotide distributes to cells of both normal and inflamed lung tissue, including alveolar macrophages, eosinophils, epithelium, blood vessel endothelium, and bronchiolar epithelium. A suitable device for the delivery of a pharmaceutical composition comprising a modified oligonucleotide includes, but is not limited to, a standard nebulizer device. Additional suitable devices include dry powder inhalers or metered dose inhalers.

In certain embodiments, pharmaceutical compositions are administered to achieve local rather than systemic exposures. For example, pulmonary administration delivers a pharmaceutical composition to the lung, with minimal systemic exposure.

Additional suitable administration routes include, but are not limited to, rectal, transmucosal, intestinal, enteral, topical, suppository, intrathecal, intraventricular, intraperitoneal, intranasal, intraocular, intramuscular, intramedullary, and intratumoral.

Conjugated Antisense Compounds

In certain embodiments, the compounds of the invention can 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.

In certain embodiments, 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 Factor VII 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 cells 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 reagent used to introduce antisense oligonucleotides into cultured cells includes Cytofectin® (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with Cytofectin® in OPTI-MEM® 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a Cytofectin® 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 Oligofectamine™ (Invitrogen Life Technologies, Carlsbad, CA). Antisense oligonucleotide is mixed with Oligofectamine™ in Opti-MEM™-1 reduced serum medium (Invitrogen Life Technologies, Carlsbad, CA) to achieve the desired concentration of oligonucleotide with an Oligofectamine™ to oligonucleotide ratio of approximately 0.2 to 0.8 μL per 100 nM.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes FuGENE 6 (Roche Diagnostics Corp., Indianapolis, IN). Antisense oligomeric compound was mixed with FuGENE 6 in 1 mL of serum-free RPMI to achieve the desired concentration of oligonucleotide with a FuGENE 6 to oligomeric compound ratio of 1 to 4 μL of FuGENE 6 per 100 nM.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation (Sambrook and Russell in *Molecular Cloning. A Laboratory Manual.* Third Edition. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York. 2001).

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 (Sambrook and Russell in *Molecular Cloning. A Laboratory Manual.* Third Edition. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York. 2001). 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 (Sambrook and Russell in *Molecular Cloning. A Laboratory Manual.* Third Edition. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York.

2001). Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE2000®, Lipofectin or Cytofectin. 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 (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). 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 Factor VII nucleic acid can be assayed in a variety of ways known in the art (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). 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.

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 GAPDH, or by quantifying total RNA using RIBOGREEN® (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A or GAPDH expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN® RNA quantification reagent (Invitrogen, Carlsbad, CA). 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 Factor VII 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).

The PCR probes can have JOE or FAM covalently linked to the 5' end and TAMRA or MGB covalently linked to the 3' end, where JOE or FAM is the fluorescent reporter dye and TAMRA or MGB is the quencher dye. In some cell types, primers and probe designed to a sequence from a different species are used to measure expression. For example, a human GAPDH primer and probe set can be used to measure GAPDH expression in monkey-derived cells and cell lines.

Gene target quantities obtained by RT, real-time PCR can be normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RiboGreen™ (Molecular Probes, Inc. Eugene, OR). GAPDH expression can be quantified by RT, real-time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA can be quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, OR).

Analysis of Protein Levels

Antisense inhibition of Factor VII nucleic acids can be assessed by measuring Factor VII protein levels. Protein levels of Factor VII 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) (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). 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 human and rat Factor VII 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 Factor VII and produce phenotypic changes, such as, increased colon length, decreased diarrhea, decreased kinin levels, reduced formation of embolism, reduced induction of asthma, reduced formation of fibrosis, reduced formation of colitis, increased time for embolism formation, increased time for asthma formation, increased time for fibrosis formation and increased time for colitis formation. Testing can be performed in normal animals, or in experimental disease models. 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 depends upon factors such as route of administration and animal body weight. In one embodiment, following a period of treatment with antisense oligonucleotides, RNA is isolated from liver tissue and changes in Factor VII nucleic acid expression are measured. Changes in Factor VII protein levels can also be measured. Changes in Factor VII expression can be measured by determing the level of inflammation, inflammatory conditions (e.g., asthma, arthritis, colitis) or inflammatory markers (inflammatory cytokines) present in the animal.

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 or is at risk for an inflammatory disease, disorder or condition. In certain embodiments, the individual is at risk for an inflammatory disease, disorder or condition as described, *supra*. In certain embodiments the invention provides methods for prophylactically reducing Factor VII 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 VII nucleic acid.

In certain embodiments, administration of a therapeutically effective amount of an antisense compound targeted to a Factor VII nucleic acid is accompanied by monitoring of Factor VII 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 Factor VII nucleic acid results in reduction of Factor VII 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 Factor VII nucleic acid results in a change in inflammatory disease, condition, symptom or marker (e.g., asthma, arthritis or colitis levels). In certain embodiments, administration of a Factor VII antisense compound increases or decreases the inflammatory disease, condition, symptom or marker 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 Factor VII are used for the preparation of a medicament for treating a patient suffering or susceptible to an inflammatory disease, disorder or condition.

In certain embodiments, the methods described herein include administering a compound comprising a modified oligonucleotide having an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobase portion complementary to Factor VII.

Certain Combination Therapies

In certain embodiments, a first agent comprising a modified oligonucleotide provided herein is co-administered with one or more secondary agents. In certain embodiments, such second agents are designed to treat the same inflammatory disease, disorder or condition as the first agent described herein. In certain embodiments, such second agents are designed to treat a different disease, disorder, or condition as the first agent described herein. In certain embodiments, such second agents are designed to treat an undesired side effect of one or more pharmaceutical compositions as described herein. In certain embodiments, such first agent are designed to treat an undesired side effect of a second agent. In certain embodiments, second agents are co-administered with the first agent to treat an undesired effect of the first agent. In certain embodiments, second agents are co-administered with the first agent to produce a combinational effect. In certain embodiments, second agents are co-administered with the first agent to produce a synergistic effect. In certain embodiments, the co-administration of the first and second agents permits use of lower dosages than would be required to achieve a therapeutic or prophylactic effect if the agents were administered as independent therapy.

In certain embodiments, a first agent and one or more second agents are administered at the same time. In certain embodiments, the first agent and one or more second agents are administered at different times. In certain embodiments, the first agent and one or more second agents are prepared together in a single pharmaceutical formulation. In certain embodiments, the first agent and one or more second agents are prepared separately.

In certain embodiments, second agents include, but are not limited to, NSAIDS and/or disease modifying drugs as described, *supra*. In certain embodiments, the disease modifying drug is administered prior to administration of the first agent. In certain embodiments, the disease modifying drug is administered following administration of the first agent. In certain embodiments the disease modifying drugs is administered at the same time as the first agent. In certain embodiments the dose of a co-administered disease modifying drug is the same as the dose that would be administered if the disease modifying drug was administered alone. In certain embodiments the dose of a co-administered disease modifying drug is lower than the dose that would be administered if the disease modifying drugs was administered alone. In certain embodiments the dose of a co-administered disease modifying drug is greater than the dose that would be administered if the disease modifying drugs was administered alone.

In certain embodiments, an antidote is administered anytime after the administration of a Factor VII specific inhibitor. In certain embodiments, an antidote is administered anytime after the administration of an antisense oligonucleotide targeting Factor VII. In certain embodiments, the antidote is administered minutes, hours, days, weeks, or months after the administration of an antisense compound targeting Factor VII. In certain embodiments, the antidote is complementary (e.g. the sense strand) to the antisense compound targeting Factor VII. In certain embodiments, the antidote is a Factor 7, Factor 7a, Factor VII, or Factor VIIa protein. In certain embodiments, the Factor 7, Factor 7a, Factor VII, or Factor VIIa protein is a human Factor 7, human Factor 7a, human Factor VII, or human Factor VIIa protein. In certain embodiments, the Factor 7 protein is NovoSeven.

ADVANTAGES OF THE INVENTION

Provided herein, for the first time, are methods and compositions for the modulation of Factor VII that can treat, prevent and/or ameliorate an inflammatory response. In a particular embodiment, provided are Factor VII oligonucleotides (oligonucleotides targeting a nucleic acid encoding Factor VII protein) to treat, prevent and/or ameliorate an inflammatory disease or condition such as arthritis or colitis or symptoms related to the disease or condition.

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 Factor VII mRNA in mouse primary hepatocytes

Antisense oligonucleotides targeted to a murine Factor VII nucleic acid were tested for their effects on Factor VII mRNA *in vitro*. Cultured mouse primary hepatocytes at a density of 32,000 cells per well were transfected using electroporation with 2,000 nM antisense

oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and mouse Factor VII mRNA levels were measured by quantitative real-time PCR using the murine primer probe set RTS2855 (forward sequence AATGAGGAACAGTGCTCCTTTGA, designated herein as SEQ ID NO: 6; reverse sequence TGTAAACAATCCAGAACTGCTTGGT, designated herein as SEQ ID NO: 7; probe sequence CCCGGGAGATCTTCAAGAGCCC, designated herein as SEQ ID NO: 8). Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN.

ISIS 403102 (SEQ ID NO: 9), which was one of the antisense oligonucleotides tested in the assay, was designed as a 5-10-5 MOE gapmer, and is 20 nucleosides 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 5 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 the gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout the gapmer are 5-methylcytosines. ISIS 403102 is targeted to nucleobases 1408 to 1427 of mouse Factor VII mRNA (GENBANK Accession No. NM_010172.3), incorporated herein as SEQ ID NO: 4. ISIS 403102 inhibited murine Factor VII mRNA by 93%.

Example 2: *In Vivo* Effect of Antisense Inhibition of Murine Factor VII in a murine colitis model

The effect of antisense oligonucleotide inhibition of Factor VII and its role in ameliorating colitis was evaluated in a dextran sulfate sodium (DSS)-induced colitis model. Administration of DSS to animals is a well known method to induce colitis and has been previously described by Okayasu et al. (Gastroenterology, 1990, 98:694-702), Cooper et al. (Lab Invest, 1993, 69:238-249) and Dieleman et al. (Clin Exp Immunol, 1998, 114:385-391).

Colitis in humans has symptoms that can include persistent diarrhea (loose, watery, or frequent bowel movements), crampy abdominal pain, fever, rectal bleeding, loss of appetite and weight loss. Pathological changes in colitis can include changes to the colon such as colon shortening (Gore, 1992, AJR, 158:59-61), formation of inflammatory lesions, diffused neutrophil infiltration, submucosa edema and muscularis propria thickening.

The effect of antisense inhibition of Factor VII by ISIS 403102 was evaluated in a murine DSS-induced colitis model.

Treatment

Swiss Webb mice (Charles River Laboratories (Wilmington, MA) were separated in groups and treated, as shown in Table 1.

Table 1

Summary of Mice Study Groups

Group (N)	ASO	DSS
1. (7)	None	No
2. (7)	None	Yes
3. (7)	FVII (403102) 60mpk	Yes

One group of 7 Swiss Webb mice was treated with 30 mg/kg of ISIS 403102 administered subcutaneously twice a week for 3 weeks (weekly dose of 60 mg/kg). Two control groups of 7 mice each were treated with PBS administered subcutaneously twice a week for 3 weeks. After the oligonucleotide treatments, 3% DSS in water was administered *ad libitum* for 6 days to the experimental group and one PBS control group.

Mice in all groups were weighed at day 0. The mice were anesthetized by constant 3% isoflurane inhalation and then euthanized by cervical dislocation on day 7 after DSS was administered. Body weights and colon lengths were measured. Liver and colon tissue was harvested for further analyses.

RNA analysis

RNA was isolated from the liver for RT-PCR analysis of Factor VII mRNA using primer probe set RTS2855. RNA levels were adjusted using mouse Cyclophilin and expressed as percent inhibition over the PBS control untreated with DSS (Group 1). Treatment with ISIS 403102 reduced Factor VII mRNA levels by 68%.

Effect on weight

The effect of treatment of ISIS 403102 on prevention of weight loss due to colitis was evaluated in the mice. Weights of the mice were taken at regular intervals and are presented in

Table 2, expressed in percent change compared to the weights at day 0. The results indicate that antisense inhibition of Factor VII led to an improvement in weights and therefore, the overall health of the mice compared to the colitis control.

Table 2

Percent change in weights compared to day 0 weights

Group (N)	ASO	DSS	% change
1	None	No	3
2	None	Yes	-5
3	FVII (403102)	Yes	-1

Effect on colon length

The entire colon was removed starting from the caecum and ending at the anus. The colons were flushed with 10 mL of PBS to remove debris. Colon length was assessed. The results are presented in Table 3, expressed in cm. Treatment with Factor VII oligonucleotide decreased the amount of colon shortening symptomatic of colitis.

Table 3

Colon length

Group (N)	ASO	DSS	Length
1	None	No	7.7
2	None	Yes	5.7
3	FVII (403102)	Yes	6.7

Effect on thrombin levels

Mice with dextran sodium sulfate (DSS)-induced colitis have elevated level of thrombin-antithrombin (TAT) complexes in blood (Anthoni, C. et al., J. Exp. Med. 204: 1595-1601, 2007) that is also observed in patients with ulcerative colitis (Kume, K. et al., Intern Med. 2007. 46: 1323-9). The mice were opened by a ventral midline incision and a 25-26 gauge needle of 1 mL syringe pre-filled with 0.6 mL sodium citrate was inserted into the vena cava. Blood was collected upto 0.6 mL and placed in a tube containing 0.7 mL of 10X contact system inhibitor cocktail (0.4 mg/mL polybrene, 0.1 M benzamidine and 2 mg/mL SBTI). The effect of antisense

inhibition of Factor VII on TAT levels in the colon was evaluated (Thrombi-Anti-Thrombin III complex, Siemens Healthcare Diagnostics, Deerfield, IL) and the results are presented in Table 4. As demonstrated, DSS administration increased TAT levels, which were decreased in a significant manner by treatment with ISIS 403102.

Table 4

Percent increase in thrombin-antithrombin (TAT) complex levels compared to the control

Group (N)	ASO	DSS	% increase
2	None	Yes	329
3	FVII (403102)	Yes	87

Effect on kinin levels

Observations on experimental models and humans with ulcerative colitis suggest a pathogenetic role of the kallikrein–kinin system in inflammatory bowel diseases (Devani, M. et al., Digestive and Liver Disease. 2005. 37: 665-673). The effect of antisense inhibition of Factor VII on kinin levels in the colon was evaluated (Phoenix Pharmaceuticals, Burlingame, CA) and the results are presented in Table 5, expressed as ng/mg colon tissue. As demonstrated, DSS administration increased bradykinin levels, which were decreased by treatment with ISIS 403102.

Table 5

Kinin levels in the colon

Group (N)	ASO	DSS	ng/mg
1	None	No	0.4
2	None	Yes	1.6
3	FVII (403102)	Yes	0.9

Example 3: *In Vivo* Effect of Antisense Inhibition of Murine Factor VII on vascular leakage in the colon of a murine colitis model

The effect of antisense oligonucleotide inhibition of Factor VII on ameliorating vascular leakage in the diseased colon was evaluated in a dextran sulfate sodium (DSS)-induced colitis model. Ulcerative colitis causes vascular leakage in the diseased colon, as seen in human

patients (Taha, Y. et al., Dig. Dis. Sci. 2004. 49: 109-115), as well as in the DSS-induced model (Jerkic, M. et al., Inflamm. Bowel Dis. 2010. 16: 1859-1870).

Treatment

Swiss Webb mice (Charles River Laboratories (Wilmington, MA) were separated in groups and treated, as shown in Table 6.

Table 6

Summary of Mice Study Groups

Group (N)	ASO	DSS
1. (5)	None	No
2. (5)	None	Yes
3. (5)	FVII (403102) **60mpk**	Yes

One group of 5 Swiss Webb mice was treated with 30 mg/kg of ISIS 403102 administered subcutaneously twice a week for 3 weeks (weekly dose of 60 mg/kg). Two control groups of 5 mice each were treated with PBS administered subcutaneously twice a week for 3 weeks. After the oligonucleotide treatments, 3% DSS in water was administered *ad libitum* for 6 days to the experimental group and one PBS control group.

All the groups were then injected with 12.5 mg/kg of Evans Blue solution. The mice were euthanized 30 min after the Evans Blue solution administration and colons were harvested and examined for permeability defects.

Evans Blue dye quantification

The full length of the colon from the caecum to the anus was removed and flushed with 10 mL of PBS to remove debris. The harvested colon tissue was placed in formamide solution overnight to leach out the Evans Blue dye. 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 colitis take up more dye and, therefore, demonstrate high OD values. As presented in Table 7, treatment with ISIS 403102 reduced vascular permeability compared to the DSS control.

Table 7

Percent increase in Evan's Blue Dye OD_{600nm} compared to the PBS control

Group (N)	ASO	DSS	% increase
2	None	Yes	137
3	FVII (403102)	Yes	57

Example 4: *In Vivo* Effect of Antisense Inhibition of Murine Factor VII on diarrhea scores of a murine colitis model

Ulcerative colitis causes severe diarrhea, as seen in human patients (Song, J. et al., Dig. Dis. Sci. 2003. 48: 417-421). The effect of antisense oligonucleotide inhibition of Factor VII on ameliorating diarrhea was evaluated in a dextran sulfate sodium (DSS)-induced colitis model.

Treatment

Swiss Webb mice (Charles River Laboratories (Wilmington, MA) were separated in groups and treated, as shown in Table 8.

Table 8

Summary of Mice Study Groups

Group (N)	ASO	DSS
1. (8)	None	No
2. (8)	None	Yes
3. (8)	FVII (403102) **60mpk**	Yes

One group of 8 Swiss Webb mice was treated with 30 mg/kg of ISIS 403102 administered subcutaneously twice a week for 3 weeks (weekly dose of 60 mg/kg). Two control groups of 8 mice each were treated with PBS administered subcutaneously twice a week for 3 weeks. After the oligonucleotide treatments, 3% DSS in water was administered *ad libitum* for 6 days to the experimental group and one PBS control group. The mice were monitored daily for manifestation of the symptoms of diarrhea.

Diarrhea score quantification

Stools were scored visually daily starting from the first day of DSS treatment. Mice were placed in the beaker with white paper towel and kept there until the first bowel movement then stools were scored. A score of 0 was given to stool which was deemed normal; score of 1 was given to soft stool; score of 2 was for diarrhea, score of 3 was given for bloody diarrhea. The maximum score given is 3. The results are presented in Table 9, and indicate that treatment with ISIS 403102 resulted in alleviation of the symptoms of diarrhea.

Table 9

Diarrhea scores

Group (N)	ASO	DSS	Day 0	Day 3	Day 4	Day 5	Day 6
1	None	No	0.0	0.0	0.0	0.0	0.0
2	None	Yes	0.0	0.3	0.4	1.5	1.6
3	FVII (403102)	Yes	0.0	0.0	0.2	0.9	1.1

Example 5: *In Vivo* Effect of Antisense Inhibition of Murine Factor VII on kallikrein activity in the colon of a murine colitis model

Activation of the kallikrein-kinin system has been implication in mediating inflammation in the active phase of ulcerative colitis (Stadnicki, A. et al., Dig. Dis. Sci. 1997. 42: 2356-2366). The effect of antisense oligonucleotide inhibition of Factor VII on kallikrein activity was evaluated in a dextran sulfate sodium (DSS)-induced colitis model.

Treatment

Swiss Webb mice (Charles River Laboratories (Wilmington, MA) were separated in groups and treated, as shown in Table 10.

Table 10

Summary of Mice Study Groups

Group (N)	ASO	DSS
1. (8)	None	No
2. (8)	None	Yes
3. (8)	FVII (403102) **60mpk**	Yes

One group of 8 Swiss Webb mice was treated with 30 mg/kg of ISIS 403102 administered subcutaneously twice a week for 3 weeks (weekly dose of 60 mg/kg). Two control groups of 8 mice each were treated with PBS administered subcutaneously twice a week for 3 weeks. After the oligonucleotide treatments, 3% DSS in water was administered *ad libitum* for 6 days to the experimental group and one PBS control group.

Quantification of kallikrein activity

A 96 well ELISA plates (MaxSorp, Nunc) were coated overnight with plasma kallikrein antibody (R&D Systems). Plasma samples from the various groups were incubated in the wells for 2 hours at room temperature to capture plasma kallikrein. After extensive washes, PBS buffer containing chromogenic substrate for plasma kallikrein (0.5mM S2308, Diapharma) was added. Amidolytic activity in samples was measured 2 hours later through quantification of absorbance at 405 nm. The results are presented as a percent change in kallikrein activity compared to the untreated PBS control. As presented in Table 11, treatment with ISIS 403102 reduced kallikrein activity compared to the PBS control.

Table 11

Percent change in kallikrein actvity compared to the PBS control

Group (N)	ASO	DSS	% change
2	None	Yes	100
3	FVII (403102)	Yes	24

Example 6: *In Vivo* Effect of Antisense Inhibition of Murine Factor VII on cytokine levels in a murine colitis model

The effect of antisense oligonucleotide inhibition of Factor VII on pro-inflammatory cytokine levels was evaluated in a dextran sulfate sodium (DSS)-induced colitis model.

Treatment

Swiss Webb mice (Charles River Laboratories (Wilmington, MA) were separated in groups and treated, as shown in Table 12.

Table 12

Summary of Mice Study Groups

Group (N)	ASO	DSS
1. (12)	None	No
2. (12)	None	Yes
3. (12)	FVII (403102) 60mpk	Yes

One group of 12 Swiss Webb mice was treated with 30 mg/kg of ISIS 403102 administered subcutaneously twice a week for 3 weeks (weekly dose of 60 mg/kg). Two control groups of 12 mice each were treated with PBS administered subcutaneously twice a week for 3 weeks. After the oligonucleotide treatments, 3% DSS in water was administered *ad libitum* for 6 days to the experimental group and one PBS control group.

Quantification of cytokine levels

The animals were euthanized by constant 3% isoflurane inhalation followed by cervical dislocation. The entire colon from the caecum to the anus was removed and flushed with 10 mL PBS to remove debris. The colons were then placed in a 15 mL polypropylene round-bottomed tube with 2 mL of cold homogenization buffer (PBS supplemented with Sigma protease cocktail S8820) and homogenized on ice with a hand homogenizer. The colon homogenates were then transferred into 2 mL Eppendorf tubes and centrifuged at 4^0C for 15 min at 15,000 g. The supernatant was carefully collected and transferred into a fresh tube. The protein concentrations of the samples were determined by Bradford assay (BioRad) and cytokine levels of IFN-γ, IL-1β, mKC, and TNF-α were analyzed using multiplex xytokine analysis kit (Meso Scale Discovery). The results are presented in Table 13 and indicate that antisense inhibition of Factor VII reduced the levels of pro-inflammatory cytokines compared to the DSS control.

Table 13

Cytokine levels

Group	ASO	DSS	IFN-γ	IL-1β	IL-6	TNF-α	mKC
1	None	No	1	6	11	2	7
2	None	Yes	21	180	223	57	13
3	FVII (403102)	Yes	9	75	60	18	8

Example 7: *In Vivo* Effect of Antisense Inhibition of Murine Factor VII in a therapeutic mouse model for asthma

The effect of antisense inhibition of Factor VII was evaluated in an ovalbumin (OVA)-induced murine model of airway hyper-responsiveness. The effect of antisense inhibition of Factor VII in treating airway hyper-responsiveness was measured by various standard assays.

Treatment #1

Treatment

BALB/c mice (Jackson Laboratories, ME) were used in a therapeutic model for airway hyper-responsiveness. The mice were 6-8 week old at the start of the studies.

In this model, ten mice were pre-sensitized by intraperitoneal injections of 20 μg OVA/alum or PBS/alum on days 0 and 14 (sensitization). All mice groups were challenged with 1% OVA in PBS intranasally between days 24, 25, and 26. A group of 5 mice were treated with 50 mg/kg of ISIS 403102 administered on days 17, 19, 21, 24 and 26. Another group of 5 mice was treated with PBS administered on days 17, 19, 21, 24 and 26. Another group of 5 mice received no treatment and were taken as the control group. The mice were analyzed on day 28.

The treatment of the various mice groups is displayed in Table 14.

Table 14

Treatment of mouse groups in a therapeutic model of airway hyper-responsiveness

Groups	Treatment	Dose (mg/kg)
1. (N = 5)	Naive	-
2. (N = 5)	Vehicle	-
3. (N = 5)	403102	50

Effect on Factor VII expression

Factor VII mRNA levels were quantified by real-time PCR assays using the primer probe set RTS2855. The treatment of ISIS 403102 resulted in 63% inhibition of Factor VII mRNA expression in the liver.

Effect on mucus production

Mucus production is a cardinal feature of bronchial asthma, leading to morbidty and mortality in the disease (Izuhara, K. et al., Curr. Med. Chem. 2009. 16: 2867-2875). The effect of antisense inhibition of Factor VII on mucus production was evaluated using a protocol from Crosby, J.R. et al. (J. Pharmacol. Exp. Ther. 2007. 321: 938-946). Lungs were inflated with 10% formalin overnight before imbedding in paraffin. Mucus cell development along the airway epithelium was assessed in paraffin-imbedded 4-m tissue sections stained with periodic acid-Schiff's reagent (PAS). The number of PAS-positive airways present in each lung section was determined. Parasagittal tissue sections were analyzed by bright field microscopy, and images were collected. Images were analyzed using Image-Pro Plus (Media Cybernetics, Silver Spring, MD) to derive an airway mucus index reflective of both the amount of mucus per airway and the number of airways affected (Karras, J.G. et al., Am. J. Respir. Cell Mol. Biol. 2007. 36: 276-285). The mucus content of all the airways per section (proximal to distal) was measured from groups of five animals. Image Pro-Plus was used to quantify the area and intensity of PAS staining per airway. The data were quantified as follows: mucus index = (average PAS staining intensity of airway epithelium) x area of airway epithelium staining with PAS)/(total area of conducting airway epithelium). This value is determined for each airway positive for mucus, and the sum of values for all positive airways is divided by the total number of airways in the lung.

The results are presented in Table 15 as a percent of the pulmonary alveolar lavage and airways. The results indicate that treatment with ISIS 403102 caused decrease in mucus production.

Table 15

Perent increase in mucus production compared to the untreated control

Groups	Treatment	% increase
2. (N = 5)	Vehicle	58
3. (N = 5)	ISIS 403102	48

Effect on eosinophil numbers

Increased eosinophil numbers are a common finding in asthma (Venge, P. Clin. Respir. J. 2010. 4: 15-19). Eosinophil granulocytes in the bronchoalveolar lavage fluid (BALF) of the mice groups are presented in Table 16, expressed as percent increase compared to the untreated

control. The results indicate that treatment with ISIS 403102 resulted in decrease in eosinophil numbers compared to the PBS control

Table 16
Eosinophil numbers

Groups	Treatment	% increase
2. (N = 5)	Vehicle	48
3. (N = 5)	403102	21

Treatment #2

Treatment

Twenty mice were pre-sensitized by intraperitoneal injections of 20 μg OVA/alum or PBS/alum on days 0 and 14 (sensitization). All mice groups were challenged with 1% OVA in PBS intranasally between days 24, 25, and 26. A group of 10 mice were treated with 50 mg/kg of ISIS 403102 administered on days 17, 19, 21, 24 and 26. Another group of 10 mice was treated with PBS administered on days 17, 19, 21, 24 and 26. Another group of 10 mice received no treatment and were taken as the control group. The mice were analyzed on day 28.

The treatment of the various mice groups is displayed in Table 17.

Table 17
Treatment of mouse groups in a therapeutic model of airway hyper-responsiveness

Groups	Treatment	Dose (mg/kg)
1. (N = 10)	Naive	-
2. (N = 10)	Vehicle	-
3. (N = 10)	403102	50

Effect on eosinophil numbers

Eosinophil granulocytes in the BALF of the mice groups are presented in Table 18, expressed as percent increase compared to the untreated control. The results indicate that treatment with ISIS 403102 resulted in decrease in eosinophil numbers compared to the PBS control.

Table 18
Eosinophil numbers

Groups	Treatment	% increase
2. (N = 10)	Vehicle	55
3. (N = 10)	403102	25

Example 8: *In Vivo* Effect of Antisense Inhibition of Murine Factor VII in a bleomycin-induced murine model of pulmonary fibrosis

The effect of antisense inhibition of Factor VII was evaluated in bleomycin-induced lung fibrosis model.

Treatment

C57BL/6 female mice 8-10 weeks old and weighing approximately 25 g were used in this experiment.

A group of 10 mice were treated with 50 mg/kg ISIS 403102 administered twice a week for six weeks. Two control groups of 10 mice each were treated with PBS administered twice a week for six weeks. At the end of week two, bleomycin at 5 U/kg was administered intrathecally to the treatment group and one of the control groups. The treatment of the various mice groups is displayed in Table 19.

Table 19
Treatment of mouse groups in a pulmonary fibrosis model

Group	Bleomycin	Treatment	Dose (mg/kg)
1. (N=10)	No	naïve	-
2. (N=10)	Yes	vehicle	-
3. (N=10)	Yes	403102	50

Effect on airway inflammatory cell recruitment

Mouse lungs were lavaged two times with 0.5 ml of PBS containing 2% fetal calf serum (FCS). BAL fluid samples were centrifuged to generate a cell pellet and a cell-free supernatant. The recovered airway cells from the cell pellet were resuspended in PBS-2% FCS, and a cytospin was performed. Cells were stained with Diff-Quik stain (Dade Behring, Newark, DE). Data are presented as the percent of each inflammatory cell type present in the total recovered

BAL cell population. The results are presented in Table 20 and indicate that antisense inhibition of Factor VII reduces the percentage of inflammatory cells recruited as a result of bleomycin-induced fibrosis.

Table 20

Airway inflammatory cell recruitment (% of total cells)

Group	Bleomycin	Treatment	Neutrophils	Lymphocytes
1. (N=10)	No	naïve	0.2	1.4
2. (N=10)	Yes	vehicle	56.0	14.0
3. (N=10)	Yes	403102	37.0	9.4

CLAIMS

What is claimed is:

1. A method for modulating an inflammatory response by administering a Factor VII specific modulator, whereby the inflammatory response is modulated

2. The method of claim 1, wherein the Factor VII specific modulator is a modified oligonucleotide targeting Factor VII.

3. A method for ameliorating an inflammatory disease, disorder or condition, in an animal comprising administering a compound targeting Factor VII to the animal, wherein the compound administered to the animal ameliorates the inflammatory disease, disorder or condition, in the animal.

4. A method for treating an animal at risk for an inflammatory disease, disorder or condition, comprising administering a therapeutically effective amount of a compound targeting Factor VII to the animal, wherein the compound administered to the animal treats the inflammatory disease, disorder or condition, in the animal.

5. A method for inhibiting Factor VII expression in an animal suffering from an inflammatory disease, disorder or condition, comprising administering a compound targeting Factor VII to the animal, wherein the compound administered to the animal inhibits Factor VII expression in the animal suffering from the inflammatory disease, disorder or condition.

6. A method for reducing the risk of inflammatory disease, disorder or condition, in an animal comprising administering a compound targeting Factor VII to the animal, wherein the compound administered to the animal reduces the risk of inflammatory disease, disorder or condition, in the animal.

7. A method comprising selecting an animal suffering from, or at risk of, an inflammatory disease, disorder or condition and administering a compound targeting Factor VII to the animal.

8. The method of claim 1, 3, 4, 5, 6, or 7 wherein Factor VII has a sequence as shown in SEQ ID NOs: 1-5.

9. The method of claim 3, 4, 5, 6, or 7, wherein the compound targeting Factor VII is a modified oligonucleotide.

10. The method of claim 2 or 9, wherein the modified oligonucleotide comprises a nucleobase sequence complementary to the nucleobase sequences recited in SEQ ID NOs: 1-5.

11. The method of claim 10, wherein the nucleobase sequence of the modified oligonucleotide is at least 90%, 95% or 100% complementary.

12. The method of claim 2 or 9, wherein the modified oligonucleotide consists of a single-stranded modified oligonucleotide.

13. The method of claim 2 or 9, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides.

14. The method of claim 13, wherein the modified oligonucleotide consists of 20 linked nucleosides.

15. The method of claim 13, wherein at least one internucleoside linkage is a modified internucleoside linkage.

16. The method of claim 15, wherein each modified internucleoside linkage is a phosphorothioate internucleoside linkage.

17. The method of claim 13, wherein at least one nucleoside comprises a modified sugar.

18. The method of claim 17, wherein at least one modified sugar is a bicyclic sugar.

19. The method of claim 17, wherein at least one modified sugar comprises a 2'-O-methoxyethyl.

20. The method of claim 13, wherein at least one nucleoside comprises a modified nucleobase.

21. The method of claim 20, wherein the modified nucleobase is a 5-methylcytosine.

22. The method of claim 2 or 9, wherein the modified oligonucleotide comprises:

(a) a gap segment consisting of linked deoxynucleosides;

(b) a 5' wing segment consisting of linked nucleosides;

(c) 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.

23. The method of claim 2 or 9, wherein the modified oligonucleotide comprises:

(a) a gap segment consisting of eight to sixteen linked deoxynucleosides;

(b) a 5' wing segment consisting of two to six linked nucleosides;

(c) a 3' wing segment consisting of two to six 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.

24. The method of claim 2 or 9, wherein the modified oligonucleotide comprises:

(a) a gap segment consisting of ten linked deoxynucleosides;

(b) a 5' wing segment consisting of five linked nucleosides;

(c) 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.

25. A method for treating an animal at risk for an inflammatory disease comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor VII nucleic acid as shown in SEQ ID NOs: 1-5, and wherein the compound administered to the animal treats the animal at risk for the inflammatory disease.

26. A method for treating an animal having an inflammatory disease comprising administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor VII nucleic acid as shown in SEQ ID NOs: 1-5, and wherein the compound administered to the animal treats the animal with the inflammatory disease.

27. The method of claim 1, 3, 4, 5, 6, 7, 25 or 26, wherein the animal is a human.

28. The method of claim 1, wherein a marker of the inflammatory response is selected from one or more of colon length, diarrhea, colon vascular permeability, mucus production, eosinophil recruitment, neutrophil recruitment, lymphocyte recruitment, kinin level, kallikrein activity or cytokine levels.

29. The method of claim 28, wherein the cytokines include INF-γ, IL-1β, IL-6, TNF-α and KC.

30. The method of claim 3, 4, 5, 6, 7, 25 or 26, wherein the inflammatory disease is colitis, diabetes, sepsis, allergic inflammation, asthma, fibrosis, antiphospholipid syndrome or graft-related disorder.

31. The method of claim 3, 4, 5, 6, 7, 25, or 26, wherein the inflammatory disease is an autoimmune disease.

32. The method of claim 30, wherein the colitis is selected from ulcerative colitis, Inflammatory Bowel Disease (IBD) and Crohn's Disease.

33. The method of claim 30, wherein the fibrosis is pulmonary fibrosis.

34. The method of claim 3, 4, 5, 6, 7, 25, or 26, wherein the inflammatory disease disorder or condition is Th1 or Th2 mediated.

35. The method of claim 34, wherein a marker for the Th1 mediated inflammatory disease disorder or condition is decreased.

36. The method of claim 35, wherein the marker for Th1 is any of the cytokines INF-γ, IL-1β, IL-6, TNF-α and KC.

37. The method of claim 34, wherein a marker for the Th2 mediated inflammatory disease disorder or condition is decreased.

38. The method of claim 37, wherein the marker for Th2 is any of eosinophil infiltration or mucus production.

39. The method of claim 28, wherein the diarrhea, colon vascular permeability, mucus production, eosinophil recruitment, neutrophil recruitment, lymphocyte recruitment, kinin level, kallikrein activity is decreased.

40. The method of claim 28, wherein shrinkage of colon length is ameliorated or prevented.

41. The method of claim 1, wherein a symptom or therapeutic endpoint of the inflammatory disease or condition is selected from one or more of colon length, diarrhea, colon vascular permeability or mucus production.

42. The method of claim 41, wherein one or more of the diarrhea, colon vascular permeability and mucus production is decreased.

43. The method of claim 41, wherein shrinkage of colon length is ameliorated or prevented.

44. The method of claim 1, 3, 4, 5, 6, 7, 25 or 26, wherein the compound is parenterally administered.

45. The method of claim 44, wherein the parenteral administration is any of subcutaneous or intravenous administration.

46. The method of claim 1, 3, 4, 5, 6, 7, 25 or 26, further comprising a second agent.

47. The method of claim 46, wherein the second agent is administered concomitantly or sequentially with the compound.

48. The method of claim 1, 3, 4, 5, 6, 7, 25 or 26, wherein the compound is a salt form.

49. The method of claim 1, 3, 4, 5, 6, 7, 25 or 26, further comprising a pharmaceutically acceptable carrier or diluent.

50. Use of a compound targeted to Factor VII for treating an inflammatory disease.

51. The use of claim 50, wherein the compound is a modified oligonucleotide.

52. The use of claim 50, wherein Factor VII has a sequence as shown in SEQ ID NOs: 1-5.

53. The method or use of claim 1, 3, 4, 5, 6, 7, 25, 26 or 50, wherein the Factor VII specific modulator or the compound targeting Factor VII is a Factor VII specific inhibitor.

54. The method or use of claim 50, wherein the Factor VII specific inhibitor is a nucleic acid, peptide, antibody or small molecule.

55. The method of or use claim 50, wherein the Factor VII specific inhibitor is a modified oligonucleotide.

56. The method or use of claim 50, wherein the Factor VII specific inhibitor decreases FVII expression.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/042307

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/7088 (2012.01)
USPC - 514/44A
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/7088, 48/00; C07H 21/04; C12N 15/11 (2012.01)
USPC - 514/44A, 44R; 536/23.1, 24.5

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0298417 A1 (FREIER et al) 25 November 2010 (25.11.2010) entire document	1-9, 25-27, 30-33, 44-56 --- 28, 29, 34-43
Y	WO 2010/121074 A1 (MONIA et al) 21 October 2010 (21.10.2010) entire document	28, 29, 34-43
A	WO 2011/008995 A1 (FREIER et al) 20 January 2011 (20.01.2010) entire document	1-9, 25-56

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
10 September 2012	02 OCT 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/042307

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

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

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

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

3. ☒ Claims Nos.: 10-24
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.