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(54) **Title**: MODULATION OF FACTOR 11 EXPRESSION

(57) **Abstract**: Disclosed herein are antisense compounds and methods for decreasing Factor 11 and treating or preventing thromboembolic complications in an individual in need thereof. Examples of disease conditions that can be ameliorated with the administration of antisense compounds targeted to Factor 11 include thrombosis, embolism, and thromboembolism, such as, deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke. Antisense compounds targeting Factor 11 can also be used as a prophylactic treatment to prevent individuals at risk for thrombosis and embolism.

MODULATION OF FACTOR 11 EXPRESSION

Cross-Reference to Related Applications

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 61/105,772, filed October 15, 2008 and U.S. Provisional Application No. 61/174,461, filed April 30, 2009. Each of the above applications is herein incorporated by reference in its entirety.

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 20091015_BIOL0107WOSEQ.txt created October 15, 2009, which is 92 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field of the Invention

Embodiments of the present invention provide methods, compounds, and compositions for reducing expression of Factor 11 mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate thromboembolic complications.

Background of the Invention

The circulatory system requires mechanisms that prevent blood loss, as well as those that counteract inappropriate intravascular obstructions. Generally, coagulation comprises a cascade of reactions culminating in the conversion of soluble fibrinogen to an insoluble fibrin gel. The steps of the cascade involve the conversion of an inactive zymogen to an activated enzyme. The active enzyme then catalyzes the next step in the cascade.

Coagulation Cascade

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).

Inhibition of coagulation.

At least three mechanisms keep the coagulation cascade in check, namely the action of activated protein C, antithrombin, and tissue factor pathway inhibitor. Activated protein C is a serine protease that degrades cofactors Va and VIIIa. Protein C is activated by thrombin with thrombomodulin, and requires coenzyme Protein S to function. Antithrombin is a serine protease inhibitor (serpin) that inhibits serine proteases: thrombin, Xa, XIIa, XIa and IXa. Tissue factor pathway inhibitor inhibits the action of Xa and the TF-VIIa complex. (Schwartz AL et al., *Trends Cardiovasc Med.* 1997; 7:234 –239.)

Disease

Thrombosis is the pathological development of blood clots, and an embolism occurs when a blood clot migrates to another part of the body and interferes with organ function. Thromboembolism may cause conditions such as deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke. Significantly, thromboembolism is a major cause of morbidity affecting over 2 million Americans every year. (Adcock *et al. American Journal of Clinical Pathology*. 1997;108:434–49). While most cases of thrombosis are due to acquired extrinsic problems, for example, surgery, cancer, immobility, some cases are due to a genetic predisposition, for example, antiphospholipid syndrome and the autosomal dominant condition, Factor V Leiden. (Bertina RM *et al. Nature* 1994; 369:64-67.)

Treatment.

The most commonly used anticoagulants, warfarin, heparin, and low molecular weight heparin (LMWH) all possess significant drawbacks.

Warfarin is typically used to treat patients suffering from atrial fibrillation. The drug interacts with vitamin K –dependent coagulation factors which include factors II, VII, IX and X. Anticoagulant proteins C and S are also inhibited by warfarin. Drug therapy using warfarin is further complicated by the fact that warfarin interacts with other medications, including drugs used to treat atrial fibrillation, such as amiodarone. Because therapy with warfarin is difficult to predict, patients must be carefully monitored in order to detect any signs of anomalous bleeding.

Heparin functions by activating antithrombin which inhibits both thrombin and factor X. (Bjork I, Lindahl U. *Mol Cell Biochem.* 1982 48: 161-182.) Treatment with heparin may cause an immunological reaction that makes platelets aggregate within blood vessels that can lead to thrombosis. This side effect is known as heparin-induced thrombocytopenia (HIT) and requires patient monitoring. Prolonged treatment with heparin may also lead to osteoporosis. LMWH can also inhibit Factor 2, but to a lesser degree than unfractioned heparin (UFH). LMWH has been implicated in the development of HIT.

Thus, current anticoagulant agents lack predictability and specificity and, therefore, require careful patient monitoring to prevent adverse side effects, such as bleeding complications. There are currently no anticoagulants which target only the intrinsic or extrinsic pathway.

Summary of the Invention

Provided herein are methods, compounds, and compositions for modulating expression of Factor 11 mRNA and protein. In certain embodiments, Factor 11 specific inhibitors modulate expression of Factor 11 mRNA and protein. In certain embodiments, Factor 11 specific inhibitors are nucleic acids, proteins, or small molecules.

In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, Factor 11 mRNA levels are reduced. In certain embodiments, Factor 11 protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such diseases, disorders, and conditions are thromboembolic complications. Such thromboembolic complications include the categories of thrombosis, embolism, and thromboembolism. In certain embodiments such thromboembolic complications include deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke.

Such diseases, disorders, and conditions can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of a thromboembolic complication include immobility, surgery (particularly orthopedic surgery), malignancy, pregnancy, older age, use of oral contraceptives, atrial fibrillation, previous thromboembolic complication, chronic inflammatory disease, and inherited or acquired prothrombotic clotting disorders. Certain outcomes associated with development of a thromboembolic complication include decreased blood flow through an affected vessel, death of tissue, and death.

In certain embodiments, methods of treatment include administering a Factor 11 specific inhibitor to an individual in need thereof.

Detailed Description of the Invention

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

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

Definitions

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

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

"2'-O-methoxyethyl" (also 2'-MOE and 2'-$O(CH_2)_2$-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.

"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 11 is an active pharmaceutical agent.

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

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

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

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

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

"Antidote compound" refers to a compound capable of decreasing the intensity or duration of any antisense-mediated 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 parallel or sequential administration.

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

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

"Contiguous nucleobases" means nucleobases immediately adjacent to each other.

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

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

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

"Factor 11 nucleic acid" or "Factor XI nucleic acid" or "F 11 nucleic acid" or "F XI nucleic acid" means any nucleic acid encoding Factor 11. For example, in certain embodiments, a Factor 11 nucleic acid includes a DNA sequence encoding Factor 11, an RNA sequence transcribed from DNA encoding Factor 11 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding Factor 11. "Factor 11 mRNA" means an mRNA encoding a Factor 11 protein.

"Factor 11 specific inhibitor" refers to any agent capable of specifically inhibiting the expression of Factor 11 mRNA and/or Factor 11 protein at the molecular level. For example, Factor 11 specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of Factor 11 mRNA and/or Factor 11 protein. In certain embodiments, by specifically modulating Factor 11 mRNA expression and/or Factor 11 protein expression, Factor 11 specific inhibitors may affect other components of the coagulation cascade including downstream components. Similarly, in certain embodiments, Factor 11 specific inhibitors may affect other molecular processes in an animal.

"Factor 11 specific inhibitor antidote" means a compound capable of decreasing the effect of a Factor 11 specific inhibitor. In certain embodiments, a Factor 11 specific inhibitor antidote is selected from a Factor 11 peptide; a Factor 11 antidote oligonucleotide, including a Factor 11 antidote compound complementary to a Factor 11 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'-deoxyribonucleosides positioned between and immediately adjacent to 5' and 3' wing segments having from one to six nucleosides.

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

"Identifying an animal at risk for thromboembolic complications" means identifying an animal having been diagnosed with a thromboembolic complication or identifying an animal predisposed to develop a thromboembolic complication. Individuals predisposed to develop a thromboembolic complication include those having one or more risk factors for thromboembolic complications including immobility, surgery (particularly orthopedic surgery), malignancy, pregnancy, older age, use of oral contraceptives, and inherited or acquired prothrombotic clotting disorders. 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.

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

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

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

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

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

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

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

"Modified sugar" refers to a substitution or change from a natural sugar.

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

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

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

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

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

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

"Nucleoside" means a nucleobase linked to a sugar.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

"Thromboembolic complication" means any disease, disorder, or condition involving an embolism caused by a thrombus. Examples of such diseases, disorders, and conditions include the categories of thrombosis, embolism, and thromboembolism. In certain embodiments, such disease disorders, and conditions include deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke.

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

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

Certain Embodiments

Embodiments of the present invention provide methods, compounds, and compositions for decreasing Factor 11 mRNA and protein expression.

Embodiments of the present invention provide methods, compounds, and compositions for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with Factor 11 in an individual in need thereof. Also contemplated are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with Factor 11. Factor 11 associated diseases, disorders, and conditions include thromboembolic complications such as thrombosis, embolism, thromboembolism, deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke.

Embodiments of the present invention provide a Factor 11 specific inhibitor for use in treating, preventing, or ameliorating a Factor 11 associated disease. In certain embodiments, Factor 11 specific inhibitors are nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of Factor 11 mRNA and/or Factor 11 protein.

In certain embodiments of the present invention, Factor 11 specific inhibitors are peptides or proteins, such as, but not limited to, alpha 1 protease inhibitors, antithrombin III, C1 inhibitors, and alpha 2 plasmin inhibitors as described in *J Clin Invest* 1982, 69:844-852; alpha 1 antitrypsin (alpha 1AT) as described in *Thromb Res* 1987, 48:145-151; Factor 11 peptide inhibitors as described in USPPN 2008/021998 and *Blood* 1998, 92:4198-206; MAP4-RGKWC as described in *Thromb Res* 2001, 104:451-465; beta 2 GPI as described in *Proc Natl Acad Sci* 2004, 101:3939-44; *Lentinus* proteinase inhibitor as described in *Eur J Biochem* 1999, 262:915-923; protease nexin-2/amyloid beta protein precursor Kunitz domain inhibitor (APPI) and antithrombin (AT) as described in *J Biol Chem* 2004, 279:29485-29492; and aprotinin as described in *J Biol Chem* 2005, 280:23523-30.

In certain embodiments of the present invention, Factor 11 specific inhibitors are antibodies, such as, but not limited to, Winston-Salem (IgG3 kappa) and Baltimore (IgG1 kappa) as described in *Blood* 1988, 72:1748-54; 5F4, 3C1, and 1F1 as described in *J Biol Chem* 1985, 260:10714-719; monoclonal antibodies as described in *Throm Haemost* 1990, 63:417-23; XI-5108 as described in *J Thromb Haem* 2006, 4:1496-1501; monoclonal antibodies 4-1 as described in *Thromb Res* 1986, 42:225-34; and abciximab antibody as described in Example 19 of USPN 6,566,140.

In certain embodiments of the present invention, Factor 11 specific inhibitors are small molecules, such as, but not limited to, diisopropyl fluorophosphates (DFP); the small molecule inhibitors as described in Examples 1-7 of USPPN 2004/0180855; and p-aminobenzamidine (pAB) as described in *J Biol Chem* 2005, 280:23523-30.

Embodiments of the present invention provide a Factor 11 specific inhibitor, as described herein, for use in treating, preventing, or ameliorating thromboembolic complications such as thrombosis, embolism, thromboembolism, deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke.

Embodiments of the present invention provide the use of Factor 11 specific inhibitors as described herein in the manufacture of a medicament for treating, ameliorating, or preventing a thromboembolic complication such as thrombosis, embolism, thromboembolism, deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke.

Embodiments of the present invention provide a Factor 11 specific inhibitor as described herein for use in treating, preventing, or ameliorating a thromboembolic complication as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Embodiments of the present invention provide the use of a Factor 11 specific inhibitor as described herein in the manufacture of a medicament for treating, preventing, or ameliorating a thromboembolic complication as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Embodiments of the present invention provide the use of a Factor 11 specific inhibitor as described herein in the manufacture of a medicament for treating, preventing, or ameliorating a thromboembolic complication as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Embodiments of the present invention provide a kit for treating, preventing, or ameliorating a thromboembolic complication as described herein wherein the kit comprises:

(i) a Factor 11 specific inhibitor as described herein; and alternatively

(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 a thromboembolic complication as described herein by combination therapy as described herein.

Embodiments of the present invention provide antisense compounds targeted to a Factor 11 nucleic acid. In certain embodiments, the Factor 11 nucleic acid is any of the sequences set forth in GENBANK Accession No. NM_000128.3 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_022792.17, truncated from 19598000 to 19624000, (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. NM_028066.1 (incorporated herein as SEQ ID NO: 6), exons 1-15 GENBANK Accession No. NW_001118167.1 (incorporated herein as SEQ ID NO: 274).

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

In certain embodiments, the compound of the invention may comprise a modified oligonucleotide comprising a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. In certain embodiments, the compound of the invention may comprise a modified oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1.

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 656 to 676 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 656 to 676 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 665 to 687 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 665 to 687 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 50% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 675 to 704 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 675 to 704 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 50% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 677 to 704 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 677 to 704 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 60% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 678 to 697 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 678 to 697 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 680 to 703 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 680 to 703 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3 and Example 30).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 683 to 702 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 683 to 702 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 90% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 738 to 759 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 738 to 759 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3 and Example 30).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 738 to 760 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 738 to 760 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 60% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 738 to 762 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 738 to 762 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 45% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1018 to 1042 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1018 to 1042 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1062 to 1089 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1089 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1062 to 1090 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1090 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 60% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1062 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 20% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1275 to 1301 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1276 to 1301 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 30).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1284 to 1308 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1291 to 1317 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nucleobases 1275 to 1318 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 16, at least 18 or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1275 to 1318 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

Embodiments of the present invention provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 10, at least 12, at least 14, at least 16, at least 18, or 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 241.

Embodiments of the present invention provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 10, at least 12, at least 14, at least 16, at least 18, or 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 269.

Embodiments of the present invention provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 10, at least 12, at least 14, at least 16, at least 18, or 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 242 to 269.

In certain embodiments, the modified oligonucleotide comprises at least 8, at least 10, at least 12, at least 14, at least 16, or at least 18 nucleobases of a nucleobase sequence selected from ISIS Nos: 22, 31, 32, 34, 36 to 38, 40, 41, 43, 51to 53, 55, 56, 59, 60, 64, 66, 71, 73, 75, 96, 98 to 103, 105 to 109, 113 to 117, 119, 124, 127, 129, 171, 172, 174, 176, 178, 179, 181 to 197, 199 to 211, and 213 to 232. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence selected from SEQ ID NOs: 22, 31, 32, 34, 36 to 38, 40, 41, 43, 51to 53, 55, 56, 59, 60, 64, 66, 71, 73, 75, 96, 98 to 103, 105 to 109, 113 to 117, 119, 124, 127, 129, 171, 172, 174, 176, 178, 179, 181 to 197, 199 to 211, and 213 to 232. In certain embodiments, the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 22, 31, 32, 34, 36 to 38, 40, 41, 43, 51to 53, 55, 56, 59, 60, 64, 66, 71, 73, 75, 96, 98 to 103, 105 to 109, 113 to 117, 119, 124, 127, 129, 171, 172, 174, 176, 178, 179, 181 to 197, 199 to 211, and 213 to 232. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the modified oligonucleotide comprises at least 8, at least 10, at least 12, at least 14, at least 16, or at least 18 nucleobases of a nucleobase sequence selected from ISIS Nos: 22, 31, 34, 37, 40, 43, 51 to 53, 60, 98, 100 to 102, 105 to 109, 114, 115, 119, 171, 174, 176, 179, 181, 186, 188 to 193, 195, 196, 199 to 210, and 213 to 232. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence selected from SEQ ID NOs: 22, 31, 34, 37, 40, 43, 51 to 53, 60, 98, 100 to 102, 105 to 109, 114, 115, 119, 171, 174, 176, 179, 181, 186, 188 to 193, 195, 196, 199 to 210, and 213 to 232. In certain embodiments, the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 22, 31, 34, 37, 40, 43, 51 to 53, 60, 98, 100 to 102, 105 to 109, 114, 115, 119, 171, 174, 176, 179, 181, 186, 188 to 193, 195, 196, 199 to 210, and 213 to 232. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the modified oligonucleotide comprises at least 8, at least 10, at least 12, at least 14, at least 16, or at least 18 nucleobases of a nucleobase sequence selected from ISIS Nos: 31, 37, 100, 105, 179, 190 to 193, 196, 202 to 207, 209, 210, 214 to 219, 221 to 224, 226, 227, 229, and 231. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence selected from SEQ ID NOs: 31, 37, 100, 105, 179, 190 to 193, 196, 202 to 207, 209, 210, 214 to 219, 221 to 224, 226, 227, 229, and 231. In certain embodiments, the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 31, 37, 100, 105, 179, 190 to 193, 196, 202 to 207, 209, 210, 214 to 219, 221 to 224, 226, 227, 229, and 231. Said modified oligonucleotide may achieve at least 90% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 3).

In certain embodiments, the modified oligonucleotide comprises at least 8, at least 10, at least 12, at least 14, at least 16, or at least 18 nucleobases of a nucleobase sequence selected from SEQ ID NOs: 34, 52, 53, 114, 115, 190, 213 to 232, 242 to 260, and 262 to 266. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence selected from SEQ ID NOs: 34, 52, 53, 114, 115, 190, 213 to 232, 242 to 260, and 262 to 266. In certain embodiments, the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 34, 52, 53, 114, 115, 190, 213 to 232, 242 to 260, and 262 to 266. Said modified oligonucleotides may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 30).

In certain embodiments, the modified oligonucleotide comprises at least 8, at least 10, at least 12, at least 14, at least 16, or at least 18 nucleobases of a nucleobase sequence selected from SEQ ID NOs: 34, 52, 53, 114, 115, 190, 213 to 216, 218 to 226, 243 to 246, 248, 249, 252 to 259, 264, and 265. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence selected from SEQ ID NOs: 34, 52, 53, 114, 115, 190, 213 to 216, 218 to 226, 243 to 246, 248, 249, 252 to 259, 264, and 265. In certain embodiments, the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 34, 52, 53, 114, 115, 190, 213 to 216, 218 to 226, 243 to 246, 248, 249, 252 to 259, 264, and 265. Said modified oligonucleotides may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 30).

In certain embodiments, the modified oligonucleotide comprises at least 8, at least 10, at least 12, at least 14, at least 16, or at least 18 nucleobases of a nucleobase sequence selected from SEQ ID NOs: 34, 190, 215, 222, 223, 226, 246, and 254. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence selected from SEQ ID NOs: 34, 190, 215, 222, 223, 226, 246, and 254. In certain embodiments, the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 34, 190, 215, 222, 223, 226, 246, and 254. Said modified oligonucleotides may achieve at least 90% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HepG2 cells (e.g. as described in Example 30).

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

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

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is 100% complementary to a nucleobase sequence of SEQ ID NO: 1 or SEQ ID NO: 2 or SEQ ID NO: 6 or SEQ ID NO: 274.

In certain embodiments, the compound has at least one modified internucleoside linkage. In certain embodiments, the internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, the compound has at least one nucleoside comprising a modified sugar. In certain embodiments, the at least one modified sugar is a bicyclic sugar. In certain embodiments, the at least one modified sugar comprises a 2'-O-methoxyethyl.

Embodiments of the present invention provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 10, at least 12, at least 14, at least 16, at least 18, or 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 241, SEQ ID NOs: 15 to 269, or SEQ ID NOs: 242 to 269, wherein at least one nucleoside comprises a modified sugar.

In certain embodiments, said at least one at least one modified sugar is a bicyclic sugar.

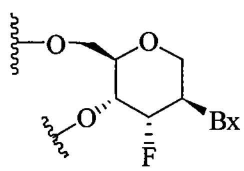
In certain embodiments, said at least one bicyclic sugar comprises a 4'- $(CH_2)_n$-O-2' bridge, wherein n is 1 or 2.

In certain embodiments, said at least one bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

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

Embodiments of the present invention provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 10, at least 12, at least 14, at least 16, at least 18, or 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 241, SEQ ID NOs: 15 to 269, or SEQ ID NOs: 242 to 269, comprising at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

In certain embodiments, said at least one tetrahydropyran modified nucleoside has the structure:



In certain embodiments, the compound 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 comprises:

(i) a gap segment consisting of linked deoxynucleosides;

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

(iii) a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar. In some such embodiments, each cytosine in the modified oligonucleotide is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide of the compound comprises:
(i) a gap segment consisting of ten linked deoxynucleosides;
(ii) a 5' wing segment consisting of five linked nucleosides;
(iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage. In some such embodiments, each cytosine in the modified oligonucleotide is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide of the compound comprises:
(i) a gap segment consisting of fourteen linked deoxynucleosides;
(ii) a 5' wing segment consisting of three linked nucleosides;
(iii) a 3' wing segment consisting of three linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage. In some such embodiments, each cytosine in the modified oligonucleotide is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide of the compound comprises:
(i) a gap segment consisting of thirteen linked deoxynucleosides;
(ii) a 5' wing segment consisting of two linked nucleosides;
(iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage. In some such embodiments, each cytosine in the modified oligonucleotide is a 5-methylcytosine.

Embodiments of the present invention provide a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 241 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

Embodiments of the present invention provide a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 269 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

Embodiments of the present invention provide a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 241 to 269 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

Embodiments of the present invention provide methods comprising administering to an animal a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 241.

Embodiments of the present invention provide methods comprising administering to an animal a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 15 to 269.

Embodiments of the present invention provide methods comprising administering to an animal a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 241 to 269.

In certain embodiments, the animal is a human.

In certain embodiments, the administering prevents deep vein thrombosis or pulmonary embolism.

In certain embodiments, the compound is co-administered with any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, and LOVENOX.

In certain embodiments, the compound is co-administered with any Factor Xa inhibitor.

In certain embodiment, the Factor Xa inhibitor is any of Rivaroxaban, LY517717, YM150, apixaban, PRT054021, and DU-176b.

In certain embodiments, the compound is administered concomitantly with any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, and LOVENOX are administered concomitantly.

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

Embodiments of the present invention provide methods comprising identifying an animal at risk for developing thromboembolic complications and administering to the at risk 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 11 nucleic acid.

In certain embodiments, the thromboembolic complication is deep vein thrombosis, pulmonary embolism, or a combination thereof.

Embodiments of the present invention provide methods comprising identifying an animal having a clotting disorder 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 11 nucleic acid.

In certain embodiments, the compound is co-administered with any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, and LOVENOX.

In certain embodiments, the compound is administered concomitantly with any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, and LOVENOX are administered concomitantly.

Embodiments of the present invention provide methods comprising reducing the risk for thromboembolic complications in an animal 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 11 nucleic acid.

Embodiments of the present invention provide methods comprising treating a clotting disorder in an animal 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 11 nucleic acid.

Embodiments of the present invention provide methods comprising inhibiting Factor 11 expression in an animal 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 11 nucleic acid.

In certain embodiments, the Factor 11 inhibition in the animal is reversed by administering an antidote to the modified oligonucleotide.

In certain embodiments, the antidote is an oligonucleotide complementary to the modified oligonucleotide.

Antisense Compounds

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

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

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

In certain embodiments anitsense oligonucleotides targeted to a Factor 11 nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a Factor 11 nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

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

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

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

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

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to a Factor 11 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 may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE, and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH2)n-O-2' bridge, where n=1 or n=2). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap segment is positioned immediately adjacent each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing segment and gap segment, or the gap segment and the 3' wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleotides. Thus, gapmers of the present invention include, but are not limited to, for example 5-10-5, 4-8-4, 4-12-3, 4-12-4, 3-14-3, 2-13-5, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1, 2-8-2, 5-8-5, or 6-8-6.

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

In certain embodiments, antisense compounds targeted to a Factor 11 nucleic acid possess a 5-10-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a Factor 11 nucleic acid possess a 3-14-3 gapmer motif.

In certain embodiments, antisense compounds targeted to a Factor 11 nucleic acid possess a 2-13-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a Factor 11 nucleic acid possess a 5-8-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a Factor 11 nucleic acid possess a 6-8-6 gapmer motif.

In certain embodiments, an antisense compound targeted to a Factor 11 nucleic acid has a gap-widened motif.

In certain embodiments, a gap-widened antisense oligonucleotide targeted to a Factor 11 nucleic acid has a gap segment of fourteen 2'-deoxyribonucleotides positioned immediately adjacent to and between wing segments of three chemically modified nucleosides. In certain embodiments, the chemical modification comprises a 2'-sugar modification. In another embodiment, the chemical modification comprises a 2'-MOE sugar modification.

In certain embodiments, a gap-widened antisense oligonucleotide targeted to a Factor 11 nucleic acid has a gap segment of thirteen 2'-deoxyribonucleotides positioned immediately adjacent to and between a 5' wing segment of two chemically modified nucleosides and a 3' wing segment of five chemically modified nucleosides. In certain embodiments, the chemical modification comprises a 2'-sugar modification. In another embodiment, the chemical modification comprises a 2'-MOE sugar modification.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode Factor 11 include, without limitation, the following: GENBANK Accession No. NM_000128.3, first deposited with GENBANK on March 24, 1999 incorporated herein as SEQ ID NO: 1; NT_022792.17, truncated from 19598000 to 19624000, first deposited with GENBANK on November 29, 2000, and incorporated herein as SEQ ID NO: 2; GENBANK Accession No. NM_028066.1, first deposited with GENBANK on June 2, 2002, incorporated herein as SEQ ID NO: 6; and exons 1-15 GENBANK Accession No. NW_001118167.1, first deposited with GENBANK on March 28, 2006, incorporated herein as SEQ ID NO: 274.

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 11 can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

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

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain emodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceeding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

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

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

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in Factor 11 mRNA levels are indicative of inhibition of Factor 11 expression. Reductions in levels of a Factor 11 protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of Factor 11 expression. For example, a prolonged aPTT time can be indicative of inhibition of Factor 11 expression. In another example, prolonged aPTT time in conjunction with a normal PT time can be indicative of inhibition of Factor 11 expression. In another example, a decreased quantity of Platelet Factor 4 (PF-4) can be indicative of inhibition of Factor 11 expression. In another example, reduced formation of thrombus or increased time for thrombus formation can be indicative of inhibition of Factor 11 expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a Factor 11 nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

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

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a Factor 11 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 11 nucleic acid).

Non-complementary nucleobases between an antisense compound and a Factor 11 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 11 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 11 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 11 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 11 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 11 nucleic acid, or specified portion thereof.

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

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

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

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

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

Modifications

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

Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

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

Modified Internucleoside Linkages

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

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

In certain embodiments, antisense compounds targeted to a Factor 11 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

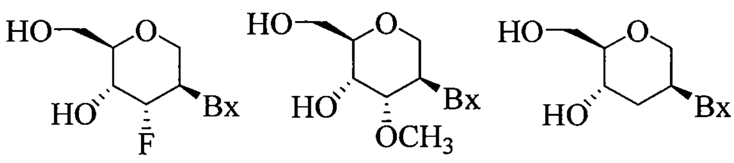
Modified Sugar Moieties

Antisense compounds of the invention can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring 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(R1)(R)2 (R = H, C1-C12 alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) 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'-OCH3 and 2'-O(CH2)2OCH3 substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C1-C10 alkyl, OCF3, O(CH2)2SCH3, O(CH2)2-O-N(Rm)(Rn), and O-CH2-C(=O)-N(Rm)(Rn), where each Rm and Rn is, independently, H or substituted or unsubstituted C1-C10 alkyl.

Examples of bicyclic nucleic acids (BNAs) 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 BNA nucleosides wherein the bridge comprises one of the formulas: 4'-(CH2)-O-2' (LNA); 4'-(CH2)-S-2'; 4'-(CH2)-O-2' (LNA); 4'-(CH2)2-O-2' (ENA); 4'-C(CH3)2-O-2' (see PCT/US2008/068922); 4'-CH(CH3)¬-O-2' and 4'-C¬H(CH2OCH3)¬-O-2' (see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-CH2-N(OCH3)-2' (see PCT/US2008/ 064591); 4'-CH2-O-N(CH3)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH2-N(R)-O-2' (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH2-C(CH3)-2'and 4'-CH2-C¬(=CH2)-2' (see PCT/US2008/ 066154); and wherein R is, independently, H, C1-C12 alkyl, or a protecting group. Each of the foregoing BNAs include various 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, nucleosides are modified by replacement of the ribosyl ring with a sugar surrogate. Such modification includes without limitation, replacement of the ribosyl ring with a surrogate ring system (sometimes referred to as DNA analogs) such as a morpholino ring, a cyclohexenyl ring, a cyclohexyl ring or a tetrahydropyranyl ring such as one having one of the formula:



Many other bicyclo and tricyclo sugar surrogate ring systems are also know in the art that can be used to modify nucleosides for incorporation into antisense compounds (see for example review article: Leumann, J. C, *Bioorganic & Medicinal Chemistry*, **2002**, *10*, 841-854). Such ring systems can undergo various additional substitutions to enhance activity.

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

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

In certain embodiments, antisense compounds targeted to a Factor 11 nucleic acid comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif.

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 ($-C{\equiv}C-CH_3$) 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 11 nucleic acid comprise one or more modified nucleobases. In certain embodiments, gap-widened antisense oligonucleotides targeted to a Factor 11 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 may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

An antisense compound targeted to a Factor 11 nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a Factor 11 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

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

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

Conjugated Antisense Compounds

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

Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of Factor 11 nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commerical vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HepG2 cells, Hep3B cells, and primary hepatocytes.

In vitro testing of antisense oligonucleotides

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

In general, cells are treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

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

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

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

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

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

RNA Isolation

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

Analysis of inhibition of target levels or expression

Inhibition of levels or expression of a Factor 11 nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitaive real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

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

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

Probes and primers are designed to hybridize to a Factor 11 nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of Factor 11 nucleic acids can be assessed by measuring Factor 11 protein levels. Protein levels of Factor 11 can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of mouse, rat, monkey, and human Factor 11 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 11 and produce phenotypic changes, such as, prolonged aPTT, prolonged aPTT time in conjunction with a normal PT, decreased quantity of Platelet Factor 4 (PF-4), and reduced formation of thrombus or increased time for thrombus formation. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, and subcutaneous. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from liver tissue and changes in Factor 11 nucleic acid expression are measured. Changes in Factor 11 protein levels are also measured using a thrombin generation assay. In addition, effects on clot times, e.g. PT and aPTT, are determined using plasma from treated animals.

Tolerability

In certain embodiments, the compounds provided herein display minimal side effects. Side effects include responses to the administration of the antisense compound that are typically unrelated to the targeting of factor 11, such as an inflammatory response in the animal. In certain embodiments compounds are well tolerated by the animal. Increased tolerability can depend on a number of factors, including, but not limited to, the nucleotide sequence of the antisense compound, chemical modifications to the nucleotides, the particular motif of unmodified and modified nucleosides in the antisense compound, or combinations thereof. Tolerability may be determined by a number of factors. Such factors include body weight, organ weight, liver function, kidney function, platelet count, white blood cell count.

In certain embodiments, the compounds provided herein demonstrate minimal effect on organ weight. In certain embodiments, the compounds demonstrate less than a 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, 2-fold or no significant increase in spleen and/or liver weight.

In certain embodiments, the compounds provided herein demonstrate minimal effect on liver function. Factors for the evaluation of liver function include ALT levels, AST levels, plasma bilirubin levels and plasma albumin levels. In certain embodiments the compounds provided herein demonstrate less than a 7-fold, less than a 6-fold, less than a 5-fold, less than a 4-fold, less than a 3-fold or less than a 2-fold or no significant increase in ALT or AST. In certain embodiments the compounds provided herein demonstrate less than a 3-fold, less than a 2-fold or no significant increase in plasma bilirubin levels.

In certain embodiments, the compounds provided herein demonstrate minimal effect on kidney function. In certain embodiments, the compounds provided herein demonstrate less than a 3-fold, less than a 2-fold, or no significant increase in plasma concentrations of blood urea nitrogen (BUN). In certain embodiments, the compounds provided herein demonstrate less than a 6-fold, 5-fold, 4-fold, 3-fold, 2-fold, or no significant increase in the ratio of urine protein to creatinine.

In certain embodiments, the compounds provided herein demonstrate minimal effect on hematological factors. In certain embodiments, the compounds provided herein demonstrate less than a 60%, 50%, 40%, 30%, 20%, 10% or 5% decrease in platelet count. In certain embodiments, the compounds provided herein demonstrate less than a 4-fold, less than a 3-fold, less than a 2-fold or no significant increase in monocyte count.

In certain embodiments compounds further display favorable pharmacokinetics. In certain embodiments, antisense compounds exhibit relatively high half-lives in relevant biological fluids or tissues.

In certain embodiments, compounds or compositions further display favorable viscosity. In certain embodiments, the viscosity of the compound or composition is no more than 40cP at a concentration of 165-185 mg/mL.

In other embodiments, the compounds display combinations of the characteristics above and reduce factor 11 mRNA expression in an animal model with high efficiency.

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 a thromboembolic complication. In certain embodiments, the individual is at risk for a blood clotting disorder, including, but not limited to, infarct, thrombosis, embolism, thromboembolism such as deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke. This includes individuals with an acquired problem, disease, or disorder that leads to a risk of thrombosis, for example, surgery, cancer, immobility, sepsis, atherosclerosis atrial fibrillation, as well as genetic predisposition, for example, antiphospholipid syndrome and the autosomal dominant condition, Factor V Leiden. In certain embodiments, the individual has been identified as in need of anticoagulation therapy. Examples of such individuals include, but are not limited to, those undergoing major orthopedic surgery (e.g., hip/knee replacement or hip fracture surgery) and patients in need of chronic treatment, such as those suffering from arterial fibrillation to prevent stroke. In certain embodiments the invention provides methods for prophylactically reducing Factor 11 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 11 nucleic acid.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to a Factor 11 nucleic acid is accompanied by monitoring of Factor 11 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 11 nucleic acid results in reduction of Factor 11 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 11 nucleic acid results in a change in a measure of blood clotting as measured by a standard test, for example, but not limited to, activated partial thromboplastin time (aPTT) test, prothrombin time (PT) test, thrombin time (TCT), bleeding time, or D-dimer. In certain embodiments, administration of a Factor 11 antisense compound increases the measure by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In some embodiments, administration of a Factor 11 antisense compound decreases the measure by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to Factor 11 are used for the preparation of a medicament for treating a patient suffering or susceptible to a thromboembolic complication.

Certain Combination Therapies

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

In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared separately.

In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include anticoagulant or antiplatelet agents. In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include NSAID/Cyclooxygenase inhibitors, such as, aspirin. In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include adenosine diphosphate (ADP) receptor inhibitors, such as, clopidogrel (PLAVIX) and ticlopidine (TICLID). In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include phosphodiesterase inhibitors, such as, cilostazol (PLETAL). In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include, glycoprotein IIB/IIIA inhibitors, such as, abciximab (REOPRO), eptifibatide (INTEGRILIN), tirofiban (AGGRASTAT), and defibrotide. In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include, adenosine reuptake inhibitors, such as, to dipyridamole (PERSANTINE). In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include, but are not limited to warfarin (and related coumarins), heparin, direct thrombin inhibitors (such as lepirudin, bivalirudin), apixaban, LOVENOX, and small molecular compounds that interfere directly with the enzymatic action of particular coagulation factors (e.g. rivaroxaban, which interferes with Factor Xa). In certain embodiments, pharmaceutical agents that may be co-administered with a Factor 11 specific inhibitor of the present invention include, but are not limited to, an additional Factor 11 inhibitor. In certain embodiments, the anticoagulant or antiplatelet agent is administered prior to administration of a pharmaceutical composition of the present invention. In certain embodiments, the anticoagulant or antiplatelet agent is administered following administration of a pharmaceutical composition of the present invention. In certain embodiments the anticoagulant or antiplatelet agent is administered at the same time as a pharmaceutical composition of the present invention. In certain embodiments the dose of a co-administered anticoagulant or antiplatelet agent is the same as the dose that would be administered if the anticoagulant or antiplatelet agent was administered alone. In certain embodiments the dose of a co-administered anticoagulant or antiplatelet agent is lower than the dose that would be administered if the anticoagulant or antiplatelet agent was administered alone. In certain embodiments the dose of a co-administered anticoagulant or antiplatelet agent is greater than the dose that would be administered if the anticoagulant or antiplatelet agent was administered alone.

In certain embodiments, the co-administration of a second compound enhances the anticoagulant effect of a first compound, such that co-administration of the compounds results in an anticoagulant effect that is greater than the effect of administering the first compound alone. In other embodiments, the co-administration results in anticoagulant effects that are additive of the effects of the compounds when administered alone. In certain embodiments, the co-administration results in anticoagulant effects that are supra-additive of the effects of the compounds when administered alone. In certain embodiments, the co-administration of a second compound increases antithrombotic activity without increased bleeding risk. In certain embodiments, the first compound is an antisense compound. In certain embodiments, the second compound is an antisense compound.

In certain embodiments, an antidote is administered anytime after the administration of a Factor 11 specific inhibitor. In certain embodiments, an antidote is administered anytime after the administration of an antisense oligonucleotide targeting Factor 11. In certain embodiments, the antidote is administered minutes, hours, days, weeks, or months after the administration of an antisense compound targeting Factor 11. In certain embodiments, the antidote is a complementary (e.g. the sense strand) to the antisense compound targeting Factor 11. In certain embodiments, the antidote is a Factor 7, Factor 7a, Factor 11, or Factor 11a protein. In certain embodiments, the Factor 7, Factor 7a, Factor 11, or Factor 11a protein is a human Factor 7, human Factor 7a, human Factor 11, or human Factor 11a protein. In certain embodiments, the Factor 7 protein is NOVOSEVEN.

Certain Co-Administered Antiplatelet Therapies

In certain embodiments, Factor 11 inhibitors are combined with antiplatelet therapies. In certain embodiments, administration of a Factor 11 inhibitor in combination with an antiplatelet therapy results in little to no appreciable or detectable increase in risk of bleeding as compared to antiplatelet therapy alone. In certain embodiments, the risk profile or risk indications are unchanged over antiplatelet therapy alone.

The combination of antiplatelet and anticoagulant therapy is used in clinical practice most frequently in patients diagnosed with, for example, thromboembolism, atrial fibrillation, a heart valve disorder, valvular heart disease, stroke, CAD, and in patients having a mechanical valve. The benefit of dual therapy relates to the probable additive effect of suppressing both platelet and coagulation factor activities. The risk of dual therapy is the potential for increased bleeding (Dowd, M. Plenary Sessions/Thrombosis Research 123 (2008)).

Prior combinations of antiplatelet and anticoagulant therapy have been shown to increase the risk of bleeding compared with anticoagulant or antiplatelet therapy alone. Such combinations include, FXa inhibitors (e.g., apixiban and rivaroxaban) with ADP receptor/P2Y12 inhibitors (Thienopyridines such as clopidogrel – also known as PLAVIX) and NSAIDs (e.g., aspirin and naproxen) (Kubitza, D. *et al.*, *Br. J. Clin. Pharmacol.* 63:4 (2006); Wong, P.C. *et al. Journal of Thrombosis and Haemostasis* 6 (2008); FDA Advisory Committee Briefing Document for New Drug Application 22-406 (2009)). For example, Wong reports that addition of certain doses of apixaban to aspirin and to aspirin plus clopidogrel produced a significant increase in bleeding time compared with aspirin alone and asprin plus clopidogrel. Kubitza reports that the combination administration of rivaroxaban and naproxen significantly increased bleeding time over naproxen alone.

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 human Factor 11 in HepG2 cells

Antisense oligonucleotides targeted to a Factor 11 nucleic acid were tested for their effects on Factor 11 mRNA *in vitro*. Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin reagent with 75 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real time PCR. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells.

The chimeric antisense oligonucleotides in Tables 1 and 2 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. Each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 1 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3) and each gapmer listed in Table 2 is targeted to SEQ ID NO: 2 (GENBANK Accession No. NT_022792.17, truncated from 19598000 to 19624000).

Table 1

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1

Oligo ID	Target Start Site	Target Stop Site	Sequence	% inhibition	SEQ ID NO
412187	38	57	TTCAAACAAGTGACATACAC	21	15
412188	96	115	TGAGAGAATTGCTTGCTTTC	21	16
412189	106	125	AAATATACCTTGAGAGAATT	8	17
412190	116	135	AGTATGTCAGAAATATACCT	24	18
412191	126	145	TTAAAATCTTAGTATGTCAG	14	19
412192	146	165	CAGCATATTTGTGAAAGTCG	44	20
412193	222	241	TGTGTAGGAAATGGTCACTT	38	21
412194	286	305	TGCAATTCTTAATAAGGGTG	80	22
412195	321	340	AAATCATCCTGAAAAGACCT	22	23
412196	331	350	TGATATAAGAAAATCATCCT	25	24
412197	376	395	ACACATTCACCAGAAACTGA	45	25
412198	550	569	TTCAGGACACAAGTAAACCA	21	26
412199	583	602	TTCACTCTTGGCAGTGTTTC	66	27
412200	612	631	AAGAATACCCAGAAATCGCT	59	28
412201	622	641	CATTGCTTGAAAGAATACCC	66	29
412202	632	651	TTGGTGTGAGCATTGCTTGA	65	30
412203	656	675	AATGTCTTTGTTGCAAGCGC	91	31
412204	676	695	TTCATGTCTAGGTCCACATA	74	32
412205	686	705	GTTTATGCCCTTCATGTCTA	69	33
412206	738	757	CCGTGCATCTTTCTTGGCAT	87	34
412207	764	783	CGTGAAAAAGTGGCAGTGGA	64	35
412208	811	830	AGACAAATGTTACGATGCTC	73	36
412209	821	840	GTGCTTCAGTAGACAAATGT	91	37
412210	896	915	TGCACAGGATTTCAGTGAAA	73	38

412211	906	925	GATTAGAAAGTGCACAGGAT	64	39
412212	1018	1037	CCGGGATGATGAGTGCAGAT	88	40
412213	1028	1047	AAACAAGCAACCGGGATGAT	71	41
412214	1048	1067	TCCTGGGAAAAGAAGGTAAA	58	42
412215	1062	1081	ATTCTTTGGGCCATTCCTGG	81	43
412216	1077	1096	AAAGATTTCTTTGAGATTCT	43	44
412217	1105	1124	AATCCACTCTCAGATGTTTT	47	45
412218	1146	1165	AACCAGAAAGAGCTTTGCTC	27	46
412219	1188	1207	GGCAGAACACTGGGATGCTG	56	47
412220	1204	1223	TGGTAAAATGAAGAATGGCA	58	48
412221	1214	1233	ATCAGTGTCATGGTAAAATG	48	49
412222	1241	1263	AACAATATCCAGTTCTTCTC	5	50
412223	1275	1294	ACAGTTTCTGGCAGGCCTCG	84	51
412224	1285	1304	GCATTGGTGCACAGTTTCTG	87	52
412225	1295	1314	GCAGCGGACGGCATTGGTGC	86	53
412226	1371	1390	TTGAAGAAAGCTTTAAGTAA	17	54
412227	1391	1410	AGTATTTTAGTTGGAGATCC	75	55
412228	1425	1444	ATGTGTATCCAGAGATGCCT	71	56
412229	1456	1475	GTACACTCATTATCCATTTT	64	57
412230	1466	1485	GATTTTGGTGGTACACTCAT	52	58
412231	1476	1495	TCCTGGGCTTGATTTTGGTG	74	59
412232	1513	1532	GGCCACTCACCACGAACAGA	80	60
412233	1555	1574	TGTCTCTGAGTGGGTGAGGT	64	61
412234	1583	1602	GTTTCCAATGATGGAGCCTC	60	62
412235	1593	1612	ATATCCACTGGTTTCCAATG	57	63
412236	1618	1637	CCATAGAAACAGTGAGCGGC	72	64
412237	1628	1647	TGACTCTACCCCATAGAAAC	48	65
412238	1642	1661	CGCAAAATCTTAGGTGACTC	71	66
412239	1673	1692	TTCAGATTGATTTAAAATGC	43	67
412240	1705	1724	TGAACCCCAAAGAAAGATGT	32	68
412241	1715	1734	TATTATTTCTTGAACCCCAA	41	69
412242	1765	1784	AACAAGGCAATATCATACCC	49	70
412243	1775	1794	TTCCAGTTTCAACAAGGCAA	70	71
412244	1822	1841	GAAGGCAGGCATATGGGTCG	53	72
412245	1936	1955	GTCACTAAGGGTATCTTGGC	75	73
412246	1992	2011	AGATCATCTTATGGGTTATT	68	74
412247	2002	2021	TAGCCGGCACAGATCATCTT	75	75
412248	2082	2101	CCAGATGCCAGACCTCATTG	53	76
412249	2195	2214	CATTCACACTGCTTGAGTTT	55	77
412250	2268	2287	TGGCACAGTGAACTCAACAC	63	78
412251	2326	2345	CTAGCATTTTCTTACAAACA	58	79
412252	2450	2469	TTATGGTAATTCTTGGACTC	39	80

412253	2460	2479	AAATATTGCCTTATGGTAAT	20	81
412254	2485	2504	TATCTGCCTATATAGTAATC	16	82
412255	2510	2529	GCCACTACTTGGTTATTTTC	38	83
412256	2564	2583	AACAAATCTATTTATGGTGG	39	84
412257	2622	2641	CTGCAAAATGGTGAAGACTG	57	85
412258	2632	2651	GTGTAGATTCCTGCAAAATG	44	86
412259	2882	2901	TTTTCAGGAAAGTGTATCTT	37	87
412260	2892	2911	CACAAATCATTTTTCAGGAA	27	88
412261	2925	2944	TCCCAAGATATTTTAAATAA	3	89
412262	3168	3187	AATGAGATAAATATTTGCAC	34	90
412263	3224	3243	TGAAAGCTATGTGGTGACAA	33	91
412264	3259	3278	CACACTTGATGAATTGTATA	27	92
413460	101	120	TACCTTGAGAGAATTGCTTG	40	93
413461	111	130	GTCAGAAATATACCTTGAGA	39	94
413462	121	140	ATCTTAGTATGTCAGAAATA	12	95
413463	381	400	GAGTCACACATTCACCAGAA	74	96
413464	627	646	GTGAGCATTGCTTGAAAGAA	42	97
413465	637	656	CTTATTTGGTGTGAGCATTG	80	98
413466	661	680	ACATAAATGTCTTTGTTGCA	79	99
413467	666	685	GGTCCACATAAATGTCTTTG	91	100
413468	671	690	GTCTAGGTCCACATAAATGT	84	101
413469	681	700	TGCCCTTCATGTCTAGGTCC	84	102
413470	692	711	GTTATAGTTTATGCCCTTCA	72	103
413471	816	835	TCAGTAGACAAATGTTACGA	67	104
413472	826	845	TGGGTGTGCTTCAGTAGACA	99	105
413473	911	930	AGCCAGATTAGAAAGTGCAC	80	106
413474	1023	1042	AGCAACCGGGATGATGAGTG	84	107
413475	1053	1072	GCCATTCCTGGGAAAAGAAG	80	108
413476	1067	1086	TTGAGATTCTTTGGGCCATT	88	109
413477	1151	1170	ACTGAAACCAGAAAGAGCTT	54	110
413478	1193	1212	AGAATGGCAGAACACTGGGA	53	111
413479	1209	1228	TGTCATGGTAAAATGAAGAA	40	112
413480	1219	1238	AAGAAATCAGTGTCATGGTA	71	113
413481	1280	1299	GGTGCACAGTTTCTGGCAGG	86	114
413482	1290	1309	GGACGGCATTGGTGCACAGT	85	115
413483	1300	1319	AACTGGCAGCGGACGGCATT	78	116
413484	1430	1449	CCTTAATGTGTATCCAGAGA	74	117
413485	1461	1480	TGGTGGTACACTCATTATCC	68	118
413486	1471	1490	GGCTTGATTTTGGTGGTACA	83	119
413487	1481	1500	AACGATCCTGGGCTTGATTT	57	120
413488	1560	1579	ACAGGTGTCTCTGAGTGGGT	49	121
413489	1588	1607	CACTGGTTTCCAATGATGGA	68	122

413490	1623	1642	CTACCCCATAGAAACAGTGA	57	123
413491	1633	1652	TTAGGTGACTCTACCCCATA	73	124
413492	1647	1666	AGACACGCAAAATCTTAGGT	68	125
413493	1710	1729	TTTCTTGAACCCCAAAGAAA	65	126
413494	1780	1799	GTGGTTTCCAGTTTCAACAA	70	127
413495	1921	1940	TTGGCTTTCTGGAGAGTATT	58	128
413496	1997	2016	GGCACAGATCATCTTATGGG	72	129
413497	2627	2646	GATTCCTGCAAAATGGTGAA	39	130
413498	2637	2656	GCAGAGTGTAGATTCCTGCA	60	131
413499	2887	2906	ATCATTTTTCAGGAAAGTGT	52	132

Table 2

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 2

Oligo ID	Target Start Site	Target Stop Site	Sequence	% inhibition	SEQ ID NO
413500	1658	1677	GTGAGACAAATCAAGACTTC	15	133
413501	2159	2178	TTAGTTTACTGACACTAAGA	23	134
413502	2593	2612	CTGCTTTATGAAAAACCAAC	22	135
413503	3325	3344	ATACCTAGTACAATGTAAAT	29	136
413504	3548	3567	GGCTTGTGTGTGGTCAATAT	54	137
413505	5054	5073	TGGGAAAGCTTTCAATATTC	57	138
413506	6474	6493	ATGGAATTGTGCTTATGAGT	57	139
413507	7590	7609	TTTCAAGCTCAGGATGGGAA	55	140
413508	7905	7924	GTTGGTAAAATGCAACCAAA	64	141
413509	8163	8182	TCAGGACACAAGTAAACCTG	66	142
413510	9197	9216	TGCAAGCTGGAAATAAAAGC	17	143
413511	9621	9640	TGCCAATTTAAAAGTGTAGC	43	144
413512	9800	9819	ATATTTCAAAATCCAGTATG	39	145
413513	9919	9938	TTCTGAATATACAAATTAAT	27	146
413514	9951	9970	TTTACTATGAAAATCTAAAT	5	147
413515	11049	11068	GGTATCCTGAGTGAGATCTA	36	148
413516	11269	11288	CCAGCTATCAGGAAAATTCC	50	149
413517	12165	12184	AAAGCTATTGGAGACTCAGA	51	150
413518	12584	12603	ATGGAATCTCTTCATTTCAT	49	151
413519	12728	12747	ATGGAGACATTCATTTCCAC	59	152
413520	13284	13303	GCTCTGAGAGTTCCAATTCA	52	153
413521	14504	14523	CTGGGAAGGTGAATTTTTAG	62	154
413522	14771	14790	TCAAGAGTCTTCATGCTACC	42	155
413523	15206	15225	TCAGTTTACCTGGGATGCTG	61	156

413524	15670	15689	GACATTATACTCACCATTAT	7	157
413525	15905	15924	GTATAAATGTGTCAAATTAA	43	158
413526	16482	16501	GTAAAGTTTTACCTTAACCT	47	159
413527	17298	17317	CCATAATGAAGAAGGAAGGG	52	160
413528	17757	17776	TTAAGTTACATTGTAGACCA	48	161
413529	18204	18223	TGTGTGGGTCCTGAAATTCT	52	162
413530	18981	19000	ATCTTGTAATTACACACCCC	27	163
413531	19174	19193	GTACACTCTGCAACAGAAGC	47	164
413532	19604	19623	AGGGAATAACATGAAGGCCC	32	165
413533	20936	20955	ATCCAGTTCACCATTGGAGA	48	166
413534	21441	21460	TTTTCCAGAAGAGACTCTTC	31	167
413535	21785	21804	GTCACATTTAAAATTTCCAA	41	168
413536	23422	23441	TTAATATACTGCAGAGAACC	37	169
413537	25893	25912	AGAAATATCCCCAGACAGAG	16	170

Example 2: Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells

Twelve gapmers, exhibiting over 84 percent or greater *in vitro* inhibition of human Factor 11, were tested at various doses in HepG2 cells. Cells were plated at a density of 10,000 cells per well and transfected using lipofectin reagent with 9.375nM, 18.75 nM, 37.5 nM, 75 nM, and 150 nM concentrations of antisense oligonucleotide, as specified in Table 3. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 (forward sequence: CAGCCTGGAGCATCGTAACA, incorporated herein as SEQ ID NO: 3; reverse sequence: TTTATCGAGCTTCGTTATTCTGGTT, incorporated herein as SEQ ID NO: 4; probe sequence: TTGTCTACTGAAGCACACCCAAACAGGGAX, incorporated herein as SEQ ID NO: 5) was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells. As illustrated in Table 3, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 3

Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells

	9.375 nM	18.75 nM	37.5 nM	75 nM	150 nM	IC_{50} (nM)	SEQ ID No.
412203	29	15	61	77	82	33	31
412206	28	44	68	80	89	22	34

412212	28	45	59	73	88	25	40
412223	33	48	62	76	81	21	51
412224	24	45	57	70	81	28	52
412225	32	42	65	78	73	23	53
413467	2	35	49	61	47	43	100
413468	14	34	56	78	75	35	101
413469	24	33	53	70	84	33	102
413476	26	44	64	73	82	25	109
413481	22	38	56	67	83	32	114
413482	26	39	59	74	82	28	115

Example 3: Antisense inhibition of human Factor 11 in HepG2 cells by oligonucleotides designed by microwalk

Additional gapmers were designed based on the gapmers presented in Table 3. These gapmers were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of the original gapmers from Table 3. Gapmers were also created with various motifs, e.g. 5-10-5 MOE, 3-14-3 MOE, and 2-13-5 MOE. These gapmers were tested *in vitro*. Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin reagent with 75 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real-time PCR. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells.

The *in vitro* inhibition data for the gapmers designed by microwalk were then compared with the *in vitro* inhibition data for the gapmers from Table 3, as indicated in Tables 4, 5, 6, 7, and 8. The oligonucleotides are displayed according to the region on the human mRNA (GENBANK Accession No. NM_000128.3) to which they map.

The chimeric antisense oligonucleotides in Table 4 were designed as 5-10-5 MOE, 3-14-3 MOE, and 2-13-5 MOE gapmers. The first listed gapmers in Table 4 are the original gapmers (see Table 3) from which the remaining gapmers were designed via microwalk and are designated by an asterisk. The 5-10-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. The 3-14-3 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 14 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 3 nucleotides each. The 2-13-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 13 2'-deoxynucleotides. The central gap is flanked on the 5' end with a wing comprising 2 nucleotides and on the 3' end with a wing comprising 5 nucleotides. For each of the motifs (5-10-5, 3-14-3, and 2-13-5), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 4 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3).

As shown in Table 4, all of the 5-10-5 MOE gapmers, 3-14-3 MOE gapmers, and 2-13-5 MOE gapmers targeted to the target region beginning at target start site 656 and ending at the target stop site 704 (i.e. nucleobases 656-704) of SEQ ID NO: 1 exhibit at least 20% inhibition of Factor 11 mRNA. Many of the gapmers exhibit at least 60% inhibition. Several of the gapmers exhibit at least 80% inhibition, including ISIS numbers: 416806, 416809, 416811, 416814, 416821, 416825, 416826, 416827, 416828, 416868, 416869, 416878, 416879, 416881, 416883, 416890, 416891, 416892, 416893, 416894, 416895, 416896, 416945, 416946, 416969, 416970, 416971, 416972, 416973, 412203, 413467, 413468, and 413469. The following ISIS numbers exhibited at least 90% inhibition: 412203, 413467, 416825, 416826, 416827, 416868, 416878, 416879, 416892, 416893, 416895, 416896, 416945, 416972, and 416973. The following ISIS numbers exhibited at least 95% inhibition: 416878, 416892, 416895, and 416896.

Table 4

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides targeted to nucleobases 656 to 704 of SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3)

ISIS No.	Target Start Site	Target Stop Site	Sequence (5' to 3')	% inhibition	Motif	SEQ ID No.
*412203	656	675	AATGTCTTTGTTGCAAGCGC	97	5-10-5	31
*413467	666	685	GGTCCACATAAATGTCTTTG	92	5-10-5	100
*413468	671	690	GTCTAGGTCCACATAAATGT	83	5-10-5	101
*413469	681	700	TGCCCTTCATGTCTAGGTCC	86	5-10-5	102
416868	656	675	AATGTCTTTGTTGCAAGCGC	93	3-14-3	31
416945	656	675	AATGTCTTTGTTGCAAGCGC	94	2-13-5	31
416806	657	676	AAATGTCTTTGTTGCAAGCG	86	5-10-5	171
416869	657	676	AAATGTCTTTGTTGCAAGCG	81	3-14-3	171

416946	657	676	AAATGTCTTTGTTGCAAGCG	86	2-13-5	171
416807	658	677	TAAATGTCTTTGTTGCAAGC	51	5-10-5	172
416870	658	677	TAAATGTCTTTGTTGCAAGC	76	3-14-3	172
416947	658	677	TAAATGTCTTTGTTGCAAGC	62	2-13-5	172
416808	659	678	ATAAATGTCTTTGTTGCAAG	55	5-10-5	173
416871	659	678	ATAAATGTCTTTGTTGCAAG	28	3-14-3	173
416948	659	678	ATAAATGTCTTTGTTGCAAG	62	2-13-5	173
416809	660	679	CATAAATGTCTTTGTTGCAA	86	5-10-5	174
416872	660	679	CATAAATGTCTTTGTTGCAA	20	3-14-3	174
416949	660	679	CATAAATGTCTTTGTTGCAA	64	2-13-5	174
416873	661	680	ACATAAATGTCTTTGTTGCA	51	3-14-3	99
416950	661	680	ACATAAATGTCTTTGTTGCA	71	2-13-5	99
416810	662	681	CACATAAATGTCTTTGTTGC	68	5-10-5	175
416874	662	681	CACATAAATGTCTTTGTTGC	49	3-14-3	175
416951	662	681	CACATAAATGTCTTTGTTGC	48	2-13-5	175
416811	663	682	CCACATAAATGTCTTTGTTG	84	5-10-5	176
416875	663	682	CCACATAAATGTCTTTGTTG	75	3-14-3	176
416952	663	682	CCACATAAATGTCTTTGTTG	51	2-13-5	176
416812	664	68	TCCACATAAATGTCTTTGTT	59	5-10-5	177
416876	664	683	TCCACATAAATGTCTTTGTT	37	3-14-3	177
416953	664	683	TCCACATAAATGTCTTTGTT	45	2-13-5	177
416813	665	684	GTCCACATAAATGTCTTTGT	70	5-10-5	178
416877	665	684	GTCCACATAAATGTCTTTGT	51	3-14-3	178
416954	665	684	GTCCACATAAATGTCTTTGT	61	2-13-5	178
416878	666	685	GGTCCACATAAATGTCTTTG	95	3-14-3	100
416955	666	685	GGTCCACATAAATGTCTTTG	75	2-13-5	100
416814	667	686	AGGTCCACATAAATGTCTTT	83	5-10-5	179
416879	667	686	AGGTCCACATAAATGTCTTT	92	3-14-3	179
416956	667	686	AGGTCCACATAAATGTCTTT	61	2-13-5	179
416815	668	687	TAGGTCCACATAAATGTCTT	63	5-10-5	180
416880	668	687	TAGGTCCACATAAATGTCTT	66	3-14-3	180
416957	668	687	TAGGTCCACATAAATGTCTT	59	2-13-5	180
416816	669	688	CTAGGTCCACATAAATGTCT	79	5-10-5	181
416881	669	688	CTAGGTCCACATAAATGTCT	81	3-14-3	181
416958	669	688	CTAGGTCCACATAAATGTCT	43	2-13-5	181
416817	670	689	TCTAGGTCCACATAAATGTC	74	5-10-5	182
416882	670	689	TCTAGGTCCACATAAATGTC	60	3-14-3	182
416959	670	689	TCTAGGTCCACATAAATGTC	25	2-13-5	182
416883	671	690	GTCTAGGTCCACATAAATGT	82	3-14-3	101
416960	671	690	GTCTAGGTCCACATAAATGT	60	2-13-5	101
416818	672	691	TGTCTAGGTCCACATAAATG	76	5-10-5	183
416884	672	691	TGTCTAGGTCCACATAAATG	69	3-14-3	183

416961	672	691	TGTCTAGGTCCACATAAATG	40	2-13-5	183
416819	673	692	ATGTCTAGGTCCACATAAAT	56	5-10-5	184
416885	673	692	ATGTCTAGGTCCACATAAAT	67	3-14-3	184
416962	673	692	ATGTCTAGGTCCACATAAAT	77	2-13-5	184
416820	674	693	CATGTCTAGGTCCACATAAA	77	5-10-5	185
416886	674	693	CATGTCTAGGTCCACATAAA	74	3-14-3	185
416963	674	693	CATGTCTAGGTCCACATAAA	48	2-13-5	185
416821	675	694	TCATGTCTAGGTCCACATAA	84	5-10-5	186
416964	675	694	TCATGTCTAGGTCCACATAA	69	2-13-5	186
412204	676	695	TTCATGTCTAGGTCCACATA	76	5-10-5	32
416888	676	695	TTCATGTCTAGGTCCACATA	76	3-14-3	32
416965	676	695	TTCATGTCTAGGTCCACATA	53	2-13-5	32
416822	677	696	CTTCATGTCTAGGTCCACAT	76	5-10-5	187
416889	677	696	CTTCATGTCTAGGTCCACAT	60	3-14-3	187
416966	677	696	CTTCATGTCTAGGTCCACAT	64	2-13-5	187
416823	678	697	CCTTCATGTCTAGGTCCACA	77	5-10-5	188
416890	678	697	CCTTCATGTCTAGGTCCACA	87	3-14-3	188
416967	678	697	CCTTCATGTCTAGGTCCACA	75	2-13-5	188
416824	679	698	CCCTTCATGTCTAGGTCCAC	64	5-10-5	189
416891	679	698	CCCTTCATGTCTAGGTCCAC	81	3-14-3	189
416968	679	698	CCCTTCATGTCTAGGTCCAC	73	2-13-5	189
416825	680	699	GCCCTTCATGTCTAGGTCCA	92	5-10-5	190
416892	680	699	GCCCTTCATGTCTAGGTCCA	100	3-14-3	190
416969	680	699	GCCCTTCATGTCTAGGTCCA	80	2-13-5	190
416893	681	700	TGCCCTTCATGTCTAGGTCC	90	3-14-3	102
416970	681	700	TGCCCTTCATGTCTAGGTCC	88	2-13-5	102
416826	682	701	ATGCCCTTCATGTCTAGGTC	94	5-10-5	191
416894	682	701	ATGCCCTTCATGTCTAGGTC	85	3-14-3	191
416971	682	701	ATGCCCTTCATGTCTAGGTC	83	2-13-5	191
416827	683	702	TATGCCCTTCATGTCTAGGT	93	5-10-5	192
416895	683	702	TATGCCCTTCATGTCTAGGT	95	3-14-3	192
416972	683	702	TATGCCCTTCATGTCTAGGT	90	2-13-5	192
416828	684	703	TTATGCCCTTCATGTCTAGG	87	5-10-5	193
416896	684	703	TTATGCCCTTCATGTCTAGG	95	3-14-3	193
416973	684	703	TTATGCCCTTCATGTCTAGG	92	2-13-5	193
416829	685	704	TTTATGCCCTTCATGTCTAG	72	5-10-5	194
416897	685	704	TTTATGCCCTTCATGTCTAG	66	3-14-3	194
416974	685	704	TTTATGCCCTTCATGTCTAG	73	2-13-5	194

The chimeric antisense oligonucleotides in Table 5 were designed as 5-10-5 MOE, 3-14-3 MOE, and 2-13-5 MOE gapmers. The first listed gapmer in Table 5 is the original gapmer (see

Table 3) from which the remaining gapmers were designed via microwalk and is designated by an asterisk. The 5-10-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. The 3-14-3 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 14 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 3 nucleotides each. The 2-13-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 13 2'-deoxynucleotides. The central gap is flanked on the 5' end with a wing comprising 2 nucleotides and on the 3' end with a wing comprising 5 nucleotides. For each of the motifs (5-10-5, 3-14-3, and 2-13-5), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 5 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3).

As shown in Table 5, all of the 5-10-5 MOE gapmers, 3-14-3 MOE gapmers, and 2-13-5 MOE gapmers targeted to the target region beginning at target start site 738 and ending at the target stop site 762 (i.e. nucleobases 738-762) of SEQ ID NO: 1 exhibit at least 45% inhibition of Factor 11 mRNA. Most of the gapmers exhibit at least 60% inhibition. Several of the gapmers exhibit at least 80% inhibition, including ISIS numbers: 412206, 416830, 416831, 416898, 416899, 416900, 416903, 416975, 416976, 416977, and 416980. The following ISIS numbers exhibited at least 90% inhibition: 412206, 416831, and 416900.

Table 5

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides targeted to nucleobases 738 to 762 of SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3)

ISIS No.	Target Start Site	Target Stop Site	Sequence (5' to 3')	% inhibition	Motif	SEQ ID No.
*412206	738	757	CCGTGCATCTTTCTTGGCAT	93	5-10-5	34
416898	738	757	CCGTGCATCTTTCTTGGCAT	88	3-14-3	34
416975	738	757	CCGTGCATCTTTCTTGGCAT	87	2-13-5	34
416830	739	758	TCCGTGCATCTTTCTTGGCA	81	5-10-5	195
416899	739	758	TCCGTGCATCTTTCTTGGCA	86	3-14-3	195
416976	739	758	TCCGTGCATCTTTCTTGGCA	83	2-13-5	195
416831	740	759	ATCCGTGCATCTTTCTTGGC	91	5-10-5	196

416900	740	759	ATCCGTGCATCTTTCTTGGC	90	3-14-3	196
416977	740	759	ATCCGTGCATCTTTCTTGGC	82	2-13-5	196
416832	741	760	CATCCGTGCATCTTTCTTGG	79	5-10-5	197
416901	741	760	CATCCGTGCATCTTTCTTGG	65	3-14-3	197
416978	741	760	CATCCGTGCATCTTTCTTGG	76	2-13-5	197
416833	742	761	TCATCCGTGCATCTTTCTTG	65	5-10-5	198
416902	742	761	TCATCCGTGCATCTTTCTTG	46	3-14-3	198
416979	742	761	TCATCCGTGCATCTTTCTTG	63	2-13-5	198
416834	743	762	GTCATCCGTGCATCTTTCTT	58	5-10-5	199
416903	743	762	GTCATCCGTGCATCTTTCTT	88	3-14-3	199
416980	743	762	GTCATCCGTGCATCTTTCTT	87	2-13-5	199

The chimeric antisense oligonucleotides in Table 6 were designed as 5-10-5 MOE, 3-14-3 MOE, and 2-13-5 MOE gapmers. The first listed gapmers in Table 6 are the original gapmers (see Table 3) from which the remaining gapmers were designed via microwalk and are designated by an asterisk. The 5-10-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. The 3-14-3 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 14 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 3 nucleotides each. The 2-13-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 13 2'-deoxynucleotides. The central gap is flanked on the 5' end with a wing comprising 2 nucleotides and on the 3' end with a wing comprising 5 nucleotides. For each of the motifs (5-10-5, 3-14-3, and 2-13-5), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 6 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3).

As shown in Table 6, all of the 5-10-5 MOE gapmers, 3-14-3 MOE gapmers, and 2-13-5 MOE gapmers targeted to the target region beginning at target start site 1018 and ending at the target stop site 1042 (i.e. nucleobases 1018-1042) of SEQ ID NO: 1 exhibit at least 80% inhibition of Factor 11 mRNA. The following ISIS numbers exhibited at least 90% inhibition: 413474, 416837, 416838, 416904, 416907, and 416908.

Table 6

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides targeted to nucleobases 1018 to 1042 of SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3)

ISIS No.	Target Start Site	Target Stop Site	Sequence (5' to 3')	% inhibition	Motif	SEQ ID No.
*412212	1018	1037	CCGGGATGATGAGTGCAGAT	89	5-10-5	40
416904	1018	1037	CCGGGATGATGAGTGCAGAT	90	3-14-3	40
416981	1018	1037	CCGGGATGATGAGTGCAGAT	87	2-13-5	40
416835	1019	1038	ACCGGGATGATGAGTGCAGA	83	5-10-5	200
416905	1019	1038	ACCGGGATGATGAGTGCAGA	85	3-14-3	200
416982	1019	1038	ACCGGGATGATGAGTGCAGA	84	2-13-5	200
416836	1020	1039	AACCGGGATGATGAGTGCAG	89	5-10-5	201
416906	1020	1039	AACCGGGATGATGAGTGCAG	88	3-14-3	201
416983	1020	1039	AACCGGGATGATGAGTGCAG	86	2-13-5	201
416837	1021	1040	CAACCGGGATGATGAGTGCA	90	5-10-5	202
416907	1021	1040	CAACCGGGATGATGAGTGCA	90	3-14-3	202
416984	1021	1040	CAACCGGGATGATGAGTGCA	89	2-13-5	202
416838	1022	1041	GCAACCGGGATGATGAGTGC	94	5-10-5	203
416908	1022	1041	GCAACCGGGATGATGAGTGC	98	3-14-3	203
416985	1022	1041	GCAACCGGGATGATGAGTGC	88	2-13-5	203
413474	1023	1042	AGCAACCGGGATGATGAGTG	93	5-10-5	107

The chimeric antisense oligonucleotides in Table 7 were designed as 5-10-5 MOE, 3-14-3 MOE, and 2-13-5 MOE gapmers. The first listed gapmer in Table 7 is the original gapmer (see Table 3) from which the remaining gapmers were designed via microwalk and is designated by an asterisk. The 5-10-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. The 3-14-3 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 14 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 3 nucleotides each. The 2-13-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 13 2'-deoxynucleotides. The central gap is flanked on the 5' end with a wing comprising 2 nucleotides and on the 3' end with a wing comprising 5 nucleotides. For each of the motifs (5-10-5, 3-14-3, and 2-13-5), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. “Target stop site” indicates the 3’-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 7 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3).

As shown in Table 7, all of the 5-10-5 MOE gapmers, 3-14-3 MOE gapmers, and 2-13-5 MOE gapmers targeted to the target region beginning at target start site 1062 and ending at the target stop site 1091 (i.e. nucleobases 1062-1091) of SEQ ID NO: 1 exhibit at least 20% inhibition of Factor 11 mRNA. Many of the gapmers exhibit at least 50% inhibition, including: 412215, 413476, 413476, 416839, 416840, 416841, 416842, 416843, 416844, 416845, 416846, 416847, 416909, 416910, 416911, 416912, 416913, 416914, 416915, 416916, 416917, 416918, 416986, 416987, 416988, 416989, 416990, 416991, 416992, 416993, 416994, 416995. The following ISIS numbers exhibited at least 80% inhibition: 412215, 413476, 413476, 416839, 416840, 416841, 416842, 416843, 416844, 416845, 416910, 416911, 416912, 416913, 416914, 416916, 416917, 416986, 416987, 416989, 416991, 416992, 416993, and 416994. The following ISIS numbers exhibited at least 90% inhibition: 413476, 413476, 416842, 416844, 416910, 416911, 416912, 416913, 416916, 416917, and 416993.

Table 7

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides targeted to nucleobases 1062 to 1091 of SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3)

ISIS No.	Target Start Site	Target Stop Site	Sequence (5' to 3')	% inhibition	Motif	SEQ ID No.
*413476	1067	1086	TTGAGATTCTTTGGGCCATT	93	5-10-5	109
412215	1062	1081	ATTCTTTGGGCCATTCCTGG	82	5-10-5	43
416909	1062	1081	ATTCTTTGGGCCATTCCTGG	78	3-14-3	43
416986	1062	1081	ATTCTTTGGGCCATTCCTGG	88	2-13-5	43
416839	1063	1082	GATTCTTTGGGCCATTCCTG	89	5-10-5	204
416910	1063	1082	GATTCTTTGGGCCATTCCTG	90	3-14-3	204
416987	1063	1082	GATTCTTTGGGCCATTCCTG	80	2-13-5	204
416840	1064	1083	AGATTCTTTGGGCCATTCCT	85	5-10-5	205
416911	1064	1083	AGATTCTTTGGGCCATTCCT	90	3-14-3	205
416988	1064	1083	AGATTCTTTGGGCCATTCCT	76	2-13-5	205
416841	1065	1084	GAGATTCTTTGGGCCATTCC	87	5-10-5	206
416912	1065	1084	GAGATTCTTTGGGCCATTCC	92	3-14-3	206
416989	1065	1084	GAGATTCTTTGGGCCATTCC	88	2-13-5	206
416842	1066	1085	TGAGATTCTTTGGGCCATTC	94	5-10-5	207
416913	1066	1085	TGAGATTCTTTGGGCCATTC	93	3-14-3	207
416990	1066	1085	TGAGATTCTTTGGGCCATTC	76	2-13-5	207

413476	1067	1086	TTGAGATTCTTTGGGCCATT	93	5-10-5	109
416914	1067	1086	TTGAGATTCTTTGGGCCATT	87	3-14-3	109
416991	1067	1086	TTGAGATTCTTTGGGCCATT	87	2-13-5	109
416843	1068	1087	TTTGAGATTCTTTGGGCCAT	89	5-10-5	208
416915	1068	1087	TTTGAGATTCTTTGGGCCAT	79	3-14-3	208
416992	1068	1087	TTTGAGATTCTTTGGGCCAT	84	2-13-5	208
416844	1069	1088	CTTTGAGATTCTTTGGGCCA	90	5-10-5	209
416916	1069	1088	CTTTGAGATTCTTTGGGCCA	91	3-14-3	209
416993	1069	1088	CTTTGAGATTCTTTGGGCCA	91	2-13-5	209
416845	1070	1089	TCTTTGAGATTCTTTGGGCC	86	5-10-5	210
416917	1070	1089	TCTTTGAGATTCTTTGGGCC	92	3-14-3	210
416994	1070	1089	TCTTTGAGATTCTTTGGGCC	83	2-13-5	210
416846	1071	1090	TTCTTTGAGATTCTTTGGGC	72	5-10-5	211
416918	1071	1090	TTCTTTGAGATTCTTTGGGC	63	3-14-3	211
416995	1071	1090	TTCTTTGAGATTCTTTGGGC	64	2-13-5	211
416847	1072	1091	TTTCTTTGAGATTCTTTGGG	50	5-10-5	212
416919	1072	1091	TTTCTTTGAGATTCTTTGGG	27	3-14-3	212
416996	1072	1091	TTTCTTTGAGATTCTTTGGG	22	2-13-5	212

The chimeric antisense oligonucleotides in Table 8 were designed as 5-10-5 MOE, 3-14-3 MOE, and 2-13-5 MOE gapmers. The first listed gapmers in Table 8 are the original gapmers (see Table 3) from which the remaining gapmers were designed via microwalk and are designated by an asterisk. The 5-10-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. The 3-14-3 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 14 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 3 nucleotides each. The 2-13-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 13 2'-deoxynucleotides. The central gap is flanked on the 5' end with a wing comprising 2 nucleotides and on the 3' end with a wing comprising 5 nucleotides. For each of the motifs (5-10-5, 3-14-3, and 2-13-5), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 8 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3).

As shown in Table 8, all of the 5-10-5 MOE gapmers, 3-14-3 MOE gapmers, and 2-13-5 MOE gapmers targeted to the target region beginning at target start site 1275 and ending at the target stop site 1318 (i.e. nucleobases 1275-1318) of SEQ ID NO: 1 exhibit at least 70% inhibition of Factor 11 mRNA. Many of the gapmers exhibit at least 80% inhibition, including: 412223, 412224, 412225, 413482, 416848, 416849, 416850, 416851, 416852, 416853, 416854, 416855, 416856, 416857, 416858, 416859, 416860, 416861, 416862, 416863, 416864, 416865, 416866, 416867, 416920, 416921, 416922, 416923, 416924, 416925, 416926, 416927, 416928, 416929, 416930, 416931, 416932, 416933, 416934, 416935, 416936, 416937, 416938, 416939, 416940, 416941, 416942, 416943, 416944, 416997, 416998, 416999, 417000, 417001, 417002, 417003, 417004, 417006, 417007, 417008, 417009, 417010, 417011, 417013, 417014, 417015, 417016, 417017, 417018, 417019, and 417020. The following ISIS numbers exhibited at least 90% inhibition: 412224, 416850, 416853, 416856, 416857, 416858, 416861, 416862, 416864, 416922, 416923, 416924, 416925, 416926, 416928, 416931, 416932, 416933, 416934, 416935, 416937, 416938, 416940, 416941, 416943, 416999, 417002, 416854, and 416859.

Table 8

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides targeted to nucleobases 1275 to 1318 of SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3)

ISIS No.	Target Start Site	Target Stop Site	Sequence (5' to 3')	% inhibition	Motif	SEQ ID No.
*412223	1275	1294	ACAGTTTCTGGCAGGCCTCG	85	5-10-5	51
*412224	1285	1304	GCATTGGTGCACAGTTTCTG	93	5-10-5	52
*413482	1290	1309	GGACGGCATTGGTGCACAGT	89	5-10-5	115
*412225	1295	1314	GCAGCGGACGGCATTGGTGC	86	5-10-5	53
416920	1275	1294	ACAGTTTCTGGCAGGCCTCG	88	3-14-3	51
416997	1275	1294	ACAGTTTCTGGCAGGCCTCG	84	2-13-5	51
416848	1276	1295	CACAGTTTCTGGCAGGCCTC	86	5-10-5	213
416921	1276	1295	CACAGTTTCTGGCAGGCCTC	88	3-14-3	213
416998	1276	1295	CACAGTTTCTGGCAGGCCTC	88	2-13-5	213
416849	1277	1296	GCACAGTTTCTGGCAGGCCT	88	5-10-5	214
416922	1277	1294	GCACAGTTTCTGGCAGGCCT	94	3-14-3	214
416999	1277	1296	GCACAGTTTCTGGCAGGCCT	92	2-13-5	214
416850	1278	1297	TGCACAGTTTCTGGCAGGCC	93	5-10-5	215
416923	1278	1297	TGCACAGTTTCTGGCAGGCC	96	3-14-3	215
417000	1278	1297	TGCACAGTTTCTGGCAGGCC	89	2-13-5	215
416851	1279	1298	GTGCACAGTTTCTGGCAGGC	88	5-10-5	216
416924	1279	1298	GTGCACAGTTTCTGGCAGGC	96	3-14-3	216
417001	1279	1298	GTGCACAGTTTCTGGCAGGC	83	2-13-5	216

416925	1280	1299	GGTGCACAGTTTCTGGCAGG	98	3-14-3	114
417002	1280	1299	GGTGCACAGTTTCTGGCAGG	92	2-13-5	114
416852	1281	1300	TGGTGCACAGTTTCTGGCAG	84	5-10-5	217
416926	1281	1300	TGGTGCACAGTTTCTGGCAG	93	3-14-3	217
417003	1281	1300	TGGTGCACAGTTTCTGGCAG	89	2-13-5	217
416853	1282	1301	TTGGTGCACAGTTTCTGGCA	91	5-10-5	218
416927	1282	1301	TTGGTGCACAGTTTCTGGCA	87	3-14-3	218
417004	1282	1301	TTGGTGCACAGTTTCTGGCA	86	2-13-5	218
416854	1283	1302	ATTGGTGCACAGTTTCTGGC	90	5-10-5	219
416928	1283	1302	ATTGGTGCACAGTTTCTGGC	91	3-14-3	219
417005	1283	1302	ATTGGTGCACAGTTTCTGGC	79	2-13-5	219
416855	1284	1303	CATTGGTGCACAGTTTCTGG	87	5-10-5	220
416929	1284	1303	CATTGGTGCACAGTTTCTGG	83	3-14-3	220
417006	1284	1303	CATTGGTGCACAGTTTCTGG	81	2-13-5	220
416930	1285	1304	GCATTGGTGCACAGTTTCTG	87	3-14-3	52
417007	1285	1304	GCATTGGTGCACAGTTTCTG	82	2-13-5	52
416856	1286	1305	GGCATTGGTGCACAGTTTCT	95	5-10-5	221
416931	1286	1305	GGCATTGGTGCACAGTTTCT	96	3-14-3	221
417008	1286	1305	GGCATTGGTGCACAGTTTCT	82	2-13-5	221
416857	1287	1306	CGGCATTGGTGCACAGTTTC	92	5-10-5	222
416932	1287	1306	CGGCATTGGTGCACAGTTTC	92	3-14-3	222
417009	1287	1306	CGGCATTGGTGCACAGTTTC	85	2-13-5	222
416858	1288	1307	ACGGCATTGGTGCACAGTTT	93	5-10-5	223
416933	1288	1307	ACGGCATTGGTGCACAGTTT	92	3-14-3	223
417010	1288	1307	ACGGCATTGGTGCACAGTTT	81	2-13-5	223
416859	1289	1308	GACGGCATTGGTGCACAGTT	90	5-10-5	224
416934	1289	1308	GACGGCATTGGTGCACAGTT	90	3-14-3	224
417011	1289	1308	GACGGCATTGGTGCACAGTT	86	2-13-5	224
416935	1290	1309	GGACGGCATTGGTGCACAGT	92	3-14-3	115
417012	1290	1309	GGACGGCATTGGTGCACAGT	72	2-13-5	115
416860	1291	1310	CGGACGGCATTGGTGCACAG	88	5-10-5	225
416936	1291	1310	CGGACGGCATTGGTGCACAG	89	3-14-3	225
417013	1291	1310	CGGACGGCATTGGTGCACAG	86	2-13-5	225
416861	1292	1311	GCGGACGGCATTGGTGCACA	92	5-10-5	226
416937	1292	1311	GCGGACGGCATTGGTGCACA	93	3-14-3	226
417014	1292	1311	GCGGACGGCATTGGTGCACA	87	2-13-5	226
416862	1293	1312	AGCGGACGGCATTGGTGCAC	90	5-10-5	227
416938	1293	1312	AGCGGACGGCATTGGTGCAC	90	3-14-3	227
417015	1293	1312	AGCGGACGGCATTGGTGCAC	87	2-13-5	227
416863	1294	1313	CAGCGGACGGCATTGGTGCA	83	5-10-5	228
416939	1294	1313	CAGCGGACGGCATTGGTGCA	88	3-14-3	228
417016	1294	1313	CAGCGGACGGCATTGGTGCA	85	2-13-5	228

416940	1295	1314	GCAGCGGACGGCATTGGTGC	92	3-14-3	53
417017	1295	1314	GCAGCGGACGGCATTGGTGC	82	2-13-5	53
416864	1296	1315	GGCAGCGGACGGCATTGGTG	93	5-10-5	229
416941	1296	1315	GGCAGCGGACGGCATTGGTG	95	3-14-3	229
417018	1296	1315	GGCAGCGGACGGCATTGGTG	82	2-13-5	229
416865	1297	1316	TGGCAGCGGACGGCATTGGT	88	5-10-5	230
416942	1297	1316	TGGCAGCGGACGGCATTGGT	85	3-14-3	230
417019	1297	1316	TGGCAGCGGACGGCATTGGT	84	2-13-5	230
416866	1298	1317	CTGGCAGCGGACGGCATTGG	88	5-10-5	231
416943	1298	1317	CTGGCAGCGGACGGCATTGG	92	3-14-3	231
417020	1298	1317	CTGGCAGCGGACGGCATTGG	84	2-13-5	231
416867	1299	1318	ACTGGCAGCGGACGGCATTG	83	5-10-5	232
416944	1299	1318	ACTGGCAGCGGACGGCATTG	83	3-14-3	232
417021	1299	1318	ACTGGCAGCGGACGGCATTG	74	2-13-5	232

Example 4: Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells

Gapmers from Example 3 (see Tables 4, 5, 6, 7, and 8), exhibiting *in vitro* inhibition of human Factor 11, were tested at various doses in HepG2 cells. Cells were plated at a density of 10,000 cells per well and transfected using lipofectin reagent with 9.375 nM, 18.75 nM, 37.5 nM and 75 nM concentrations of antisense oligonucleotide, as specified in Table 9. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells. As illustrated in Table 9, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 9

Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells via transfection of oligonucleotides with lipofectin

	9.375 nM	18.75 nM	37.5 nM	75 nM	Motif	IC_{50} (nM)	SEQ ID No.
412203	33	40	62	74	5-10-5	24	31
412206	24	47	69	86	5-10-5	21	34
413467	35	51	62	69	5-10-5	20	100
413474	29	44	57	67	5-10-5	28	107
413476	24	58	62	77	5-10-5	21	109

416825	23	52	73	92	5-10-5	20	190
416826	8	36	58	84	5-10-5	29	191
416827	31	42	62	77	5-10-5	23	192
416838	31	51	64	86	5-10-5	19	203
416842	18	33	62	71	5-10-5	31	207
416850	4	30	67	84	5-10-5	29	215
416856	21	45	58	74	5-10-5	27	221
416858	0	28	54	82	5-10-5	33	223
416864	18	43	62	78	5-10-5	26	229
416878	22	34	60	82	5-10-5	27	100
416892	16	50	70	85	3-14-3	23	190
416895	39	57	66	71	3-14-3	15	192
416896	22	39	57	81	3-14-3	27	193
416908	36	57	67	76	3-14-3	16	203
416922	14	25	49	75	3-14-3	36	214
416923	36	47	60	67	3-14-3	23	215
416924	25	38	56	59	3-14-3	36	216
416925	13	38	59	75	3-14-3	30	114
416926	31	43	63	82	3-14-3	22	217
416931	44	39	57	71	3-14-3	22	221
416941	33	54	63	78	3-14-3	19	229
416945	34	45	62	65	2-13-5	24	31
416969	17	39	61	76	2-13-5	28	190
416972	32	40	60	69	2-13-5	26	192
416973	60	75	85	87	2-13-5	3	193
416984	26	50	62	81	2-13-5	22	202
416985	17	30	47	57	2-13-5	49	203
416989	18	41	62	83	2-13-5	26	206
416993	15	37	50	68	2-13-5	36	209
416999	24	37	55	73	2-13-5	30	214
417000	35	47	58	70	2-13-5	23	215
417002	35	52	67	70	2-13-5	19	114
417003	26	44	60	56	2-13-5	33	217

The gapmers were also transfected via electroporation and their dose dependent inhibition of human Factor 11 mRNA was measured. Cells were plated at a density of 20,000 cells per well and transfected via electroporation with 0.7 μM, 2.2 μM, 6.7 μM, and 20 μM concentrations of antisense oligonucleotide, as specified in Table 10. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells. As illustrated in Table 10, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 10

Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells via transfection of oligonucleotides with electroporation

	0.7 µM	2.2 µM	6.7 µM	20 µM	IC_{50} (µM)	SEQ ID No.
412203	11	60	70	91	2.7	31
412206	22	39	81	94	2.7	34
413467	5	31	65	89	4.2	100
413474	0	5	52	81	6.9	107
413476	40	69	88	93	0.9	109
416825	27	74	92	98	1.3	190
416826	2	47	86	82	3.2	191
416827	37	68	87	92	1.1	192
416838	5	30	55	83	5.1	203
416842	0	10	66	92	5.0	207
416850	14	25	81	91	3.4	215
416856	0	29	47	93	5.1	221
416858	5	20	56	86	5.3	223
416864	32	65	78	90	1.4	229
416878	1	26	75	85	4.3	100
416892	14	52	82	92	2.5	190
416895	0	62	70	91	3.0	192
416896	12	35	81	89	3.2	193
416908	7	58	74	89	2.8	203
416922	35	51	77	91	1.7	214
416923	15	30	60	90	4.0	215
416924	22	40	63	70	4.1	216
416925	0	40	76	80	3.9	114
416926	47	71	91	94	0.6	217
416931	7	24	60	82	5.1	221
416941	16	38	79	89	3.0	229
416945	48	70	81	88	0.6	31
416969	25	34	86	92	2.5	190
416972	25	30	48	88	4.3	192
416973	20	48	86	93	2.3	193
416984	43	54	88	90	1.1	202
416985	12	48	45	69	5.8	203

416989	32	65	88	94	1.3	206
416993	22	48	87	92	2.2	209
416999	20	42	77	88	2.8	214
417000	46	73	76	89	0.6	215
417002	32	38	82	91	2.2	114
417003	0	34	75	89	3.9	217

Example 5: Selection and confirmation of effective dose-dependent antisense inhibition of human Factor 11 in HepG2 cells

Gapmers exhibiting significant dose-dependent inhibition of human Factor 11 in Example 4 were selected and tested at various doses in HepG2 cells. Cells were plated at a density of 10,000 cells per well and transfected using lipofectin reagent with 2.34 nM, 4.69 nM, 9.375 nM, 18.75 nM, 37.5 nM, and 75 nM concentrations of antisense oligonucleotide, as specified in Table 11. After a treatment period of approximately 16 hours, RNA was isolated from the cells and human Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of human Factor 11, relative to untreated control cells. As illustrated in Table 11, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells compared to the control.

Table 11

Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells via transfection of oligonucleotides with lipofectin

	2.34 nM	4.69 nM	9.375 nM	18.75 nM	37.5 nM	75 nM	Motif	IC50 (nM)	SEQ ID No.
416825	4	22	39	57	79	89	5-10-5	13	190
416826	15	22	32	54	76	90	5-10-5	15	191
416838	21	37	50	63	74	83	5-10-5	10	203
416850	24	31	49	55	70	77	5-10-5	13	215
416858	11	35	46	61	75	77	5-10-5	11	223
416864	13	34	42	65	68	80	5-10-5	15	229
416892	14	34	49	70	84	93	3-14-3	9	190
416925	24	34	45	56	67	72	3-14-3	13	114
416999	10	26	42	62	72	80	2-13-5	14	214
417002	17	26	49	61	81	84	2-13-5	12	114
417003	6	29	48	64	73	82	2-13-5	11	217

The gapmers were also transfected via electroporation and their dose dependent inhibition of human Factor 11 mRNA was measured. Cells were plated at a density of 20,000 cells per well and transfected via electroporation with 625 nM, 1250 nM, 2500 nM, 5,000 nM, 10,000 nM, and 20,000 nM concentrations of antisense oligonucleotide, as specified in Table 12. After a treatment period of approximately 16 hours, RNA was isolated from the cells and human Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of human Factor 11, relative to untreated control cells. As illustrated in Table 12, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells compared to the control.

Table 12

Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells via transfection of oligonucleotides with electroporation

	625 nM	1250 nM	2500 nM	5000 nM	10000 nM	20000 nM	IC50 (μM)	SEQ ID No.
416825	69	84	91	94	96	97	19	190
416826	67	82	89	92	95	97	33	191
416838	66	79	87	90	93	96	43	203
416850	69	80	87	90	93	96	25	215
416858	65	77	87	89	93	93	44	223
416864	45	74	84	87	92	94	338	229
416892	66	86	96	97	100	100	31	190
416925	64	80	88	91	95	96	51	114
416999	61	82	89	94	94	97	67	214
417002	59	72	86	90	94	96	156	114
417003	60	74	86	90	95	95	123	217

Example 6: Selection and confirmation of effective dose-dependent antisense inhibition of human Factor 11 in cyano primary hepatocytes

Gapmers from Example 4 exhibiting significant dose dependent *in vitro* inhibition of human Factor 11 were also tested at various doses in cyano primary hepatocytes. Cells were plated at a density of 35,000 cells per well and transfected via electroporation with 0.74 nM, 2.2 nM, 6.7 nM, 20 nM, 60 nM, and 180 nM concentrations of antisense oligonucleotide, as specified in Table 13. After a treatment period of approximately 16 hours, RNA was isolated from the cells and human Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of human Factor 11, relative to untreated control cells. As illustrated in Table 13, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells compared to the control.

Table 13

Dose-dependent antisense inhibition of human Factor 11 in cyano primary hepatocytes

	0.74 nM	2.2 nM	6.7 nM	20 nM	60 nM	180 nM	IC_{50} (μM)	SEQ ID No.
416825	5	22	51	61	77	84	1.0	190
416826	13	24	34	67	69	71	1.3	191
416838	0	0	21	34	48	62	6.9	203
416850	2	20	24	65	69	67	1.6	215
416858	2	13	22	44	63	68	3.7	223
416864	0	1	15	23	47	64	7.7	229
416892	20	20	43	62	88	92	1.0	190
416925	0	9	1	48	55	76	4.4	114
416999	3	40	36	62	67	82	1.3	214
417002	32	16	28	38	55	71	4.0	114
417003	12	18	19	39	58	74	4.1	217

Example 7: Selection and confirmation of effective dose -dependent antisense inhibition of human Factor 11 in HepB3 cells by gapmers

Gapmers exhibiting *in vitro* inhibition of human Factor 11 in Example 4 were tested at various doses in human HepB3 cells. Cells were plated at a density of 4,000 cells per well and transfected using lipofectin reagent with 2.3 nM, 4.7 nM, 9.4 nM, 18.75 nM, 37.5 nM, and 75 nM concentrations of antisense oligonucleotide, as specified in Table 14. After a treatment period of approximately 16 hours, RNA was isolated from the cells and human Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells. As illustrated in Table 14, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells compared to the control.

Table 14

Dose-dependent antisense inhibition of human Factor 11 in HepB3 cells

ISIS No.	2.3 nM	4.7 nM	9.4 nM	18.75 nM	37.5 nM	75 nM	IC_{50} (nM)	SEQ ID No.
416825	0	15	34	36	53	59	35	190
416826	16	28	38	55	64	66	16	191
416838	23	34	43	59	71	56	11	203
416850	22	32	43	56	75	60	13	215
416858	17	34	43	57	74	62	12	223
416864	24	37	42	66	76	63	9	229
416892	28	34	50	68	82	72	9	190
416925	26	33	45	59	72	60	12	114
416999	19	33	42	60	71	59	12	214
417002	24	30	46	57	71	65	13	114
417003	11	28	40	40	63	58	17	217

The gapmers were also transfected via electroporation and their dose dependent inhibition of human Factor 11 mRNA was measured. Cells were plated at a density of 20,000 cells per well and transfected via electroporation with 41.15 nM, 123.457 nM, 370.37 nM, 1111.11 nM, 3333.33 nM, and 10,000 nM concentrations of antisense oligonucleotide, as specified in Table 15. After a treatment period of approximately 16 hours, RNA was isolated from the cells and human Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of human Factor 11, relative to untreated control cells. As illustrated in Table 15, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells compared to the control.

Table 15

Dose-dependent antisense inhibition of human Factor 11 in HepB3 cells

	41.15 nM	123.457 nM	370.37 nM	1111.11 nM	3333.33 nM	10000 nM	IC_{50} (μM)	SEQ ID No.
416825	32	40	48	75	90	92	0.16	190
416826	0	0	34	61	87	92	0.78	191
416838	12	9	28	40	77	88	1.20	203

416850	26	38	51	73	90	95	0.30	215
416858	23	45	52	64	87	92	0.30	223
416864	4	3	6	35	75	87	2.20	229
416892	9	12	28	65	89	98	0.61	190
416925	27	39	50	73	88	96	0.20	114
416999	31	45	62	78	94	97	0.16	214
417002	19	0	31	47	86	93	1.20	114
417003	31	0	15	43	84	92	1.50	217

Example 8: Antisense inhibition of murine Factor 11 in primary mouse hepatocytes

Chimeric antisense oligonucleotides targeting murine Factor 11 were designed as 5-10-5 MOE gapmers targeting murine Factor 11 (GENBANK Accession No. NM_028066.1, incorporated herein as SEQ ID NO: 6). The gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. Each nucleotide in each wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gaper are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. The antisense oligonucleotides were evaluated for their ability to reduce murine Factor 11 mRNA in primary mouse hepatocytes.

Primary mouse hepatocytes were treated with 6.25 nM, 12.5 nM, 25 nM, 50 nM, 100 nM, and 200 nM of antisense oligonucleotides for a period of approximately 24 hours. RNA was isolated from the cells and murine Factor 11 mRNA levels were measured by quantitative real-time PCR. Murine Factor 11 primer probe set RTS 2898 (forward sequence ACATGACAGGCGCGATCTCT, incorporated herein as SEQ ID NO: 7; reverse sequence TCTAGGTTCACGTACACATCTTTGC, incorporated herein as SEQ ID NO: 8; probe sequence TTCCTTCAAGCAATGCCCTCAGCAATX, incorporated herein as SEQ ID NO: 9) was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN. Several of the murine antisense oligonucleotides reduced Factor 11 mRNA levels in a dose-dependent manner.

Example 9: Cross-reactive antisense inhibition of murine Factor 11 in primary mouse hepatocytes

Antisense oligonucleotides targeted to a murine factor 11 nucleic acid were tested for their effects on Factor 11 mRNA *in vitro*. Cultured primary mouse hepatocytes at a density of 10,000 cells per well were treated with 100 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and mouse Factor 11 mRNA levels were measured by quantitative real-time PCR. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells.

The chimeric antisense oligonucleotides in Tables 16 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleotides each. Each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Mouse target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Mouse target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. All the mouse oligonucleotides listed show cross-reactivity between the mouse Factor 11 mRNA (GENBANK Accession No. NM_028066.1), incorporated herein as SEQ ID NO: 6 and the human Factor 11 mRNA (GENBANK Accession No. NM_000128.3), incorporated herein as SEQ ID NO: 1. "Human Target Start Site" indicates the 5'-most nucleotide in the human mRNA (GENBANK Accession No. NM_000128.3) to which the antisense oligonucleotide is targeted. "Human Target Stop Site" indicates the 3'-most nucleotide in the human mRNA (GENBANK Accession No. NM_000128.3) to which the antisense oligonucleotide is targeted. "Number of mismatches" indicates the mismatches between the mouse oligonucleotide and the human mRNA sequence.

Table 16
Inhibition of mouse Factor 11 mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 6

ISIS No	Mouse Target Start Site	Mouse Target Stop Site	Sequence (5' to 3')	% Inhibition	SEQ ID No.	Human Target Start Site	Human Target Stop Site	No. of mismatches
404050	379	398	TGCTTGAAGGAATATCCAGA	82	233	619	638	2
404054	448	467	TAGTTCATGCCCTTCATGTC	45	234	688	707	1
404055	453	472	TGTTATAGTTCATGCCCTTC	27	235	693	712	1
404066	686	705	AATGTCCCTGATACAAGCCA	37	236	926	945	1
404067	691	710	GGGAAAATGTCCCTGATACA	39	237	931	950	1
404083	1299	1318	TGTGCAGAGTCACCTGCCAT	47	238	1533	1552	2
404087	1466	1485	TTCTTGAACCCTGAAGAAAG	29	239	1709	1728	2
404089	1477	1496	TGAATTATCATTTCTTGAAC	6	240	1720	1739	2
404090	1483	1502	TGATCATGAATTATCATTTC	42	241	1726	1745	2

Example 10: *In vivo* antisense inhibition of murine Factor 11

Several antisense oligonucleotides targeted to murine Factor 11 mRNA (GENBANK Accession No. NM_028066.1, incorporated herein as SEQ ID NO: 6) showing statistically significant dose-dependent inhibition were evaluated *in vivo*. BALB/c mice were treated with ISIS 404057 (TCCTGGCATTCTCGAGCATT, target start site 487, incorporated herein as SEQ ID NO: 10) and ISIS 404071 (TGGTAATCCACTTTCAGAGG, target start site 869, incorporated herein as SEQ ID NO: 11).

Treatment

BALB/c mice were injected with 5 mg/kg, 10 mg/kg, 25 mg/kg, or 50 mg/kg of ISIS 404057 or ISIS 404071 twice a week for 3 weeks. A control group of mice was injected with phosphate buffered saline (PBS) twice a week for 3 weeks. Mice were sacrificed 5 days after receiving the last dose. Whole liver was harvested for RNA analysis and plasma was collected for clotting analysis (PT and aPTT) and protein analysis.

RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of Factor 11. As shown in Table 17, the antisense oligonucleotides achieved dose-dependent reduction of murine Factor 11 over the PBS control. Results are presented as percent inhibition of Factor 11, relative to control.

Table 17
Dose-dependent antisense inhibition of murine Factor 11 mRNA in BALB/c mice

	mg/kg	% inhibition
404057	5	40
	10	64
	25	85
	50	95
404071	5	72
	10	82
	25	93
	50	96

PT and aPTT assay

Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) were measured using platelet poor plasma (PPP) from mice treated with ISIS 404057 and ISIS 404071. PT and aPTT values provided in Table 18 are reported as International Normalized Ratio (INR) values. INR values for PT and aPTT were determined by dividing the PT or aPTT value for each experimental group (i.e. 5 mg/kg, 10 mg/kg, 25 mg/kg, and 50 mg/kg treatment with ISIS 404057 or ISIS 404071) by the PT or aPTT for the PBS treated group. This ratio was then raised to the power of the International Sensitivity Index (ISI) of the tissue factor used. As shown in Table 18, PT was not significantly prolonged in mice treated with ISIS 404057 or ISIS 404071. However, aPTT was prolonged in a dose-dependent manner in mice treated with ISIS 404057 and ISIS 404071. These data suggest that antisense reduction of Factor 11 affects the contact activation pathway, but not the extrinsic pathway of blood coagulation.

Table 18
Effect of ISIS 404071 and 404057 on PT and aPTT in BALB/c mice

	Dose in mg/kg	PT INR	aPTT INR
ISIS 404057	5	1.00	1.07

	10	0.94	1.19
	25	1.02	1.27
	50	1.00	1.37
ISIS 404071	5	1.06	1.09
	10	1.08	1.13
	25	1.06	1.35
	50	1.02	2.08

Protein Analysis

Factor 11 proenzyme from the plasma of mice treated with ISIS 404071, was measured using a F11 assay based on clotting time. Clotting times were determined in duplicate with a ST4 semi-automated coagulation instrument (Diagnostica Stago, NJ). Thirty μl of citrated sample plasma diluted 1/20 in HEPES-NaCl buffer with BSA was incubated with 30μl aPTT reagent (Platelet Factor 3 reagent plus particulate activator) and 30μl of citrated plasma deficient of Factor 11 (human congential, George King Bio-Medical Inc.) at 37°C to initiate clotting. Results were interpolated on a standard curve of serially diluted citrated control murine plasma.

As shown in Table 19, treatment with ISIS 404071 resulted in a significant dose-dependent reduction of Factor 11 protein. Results are presented as percent inhibition of Factor 11, relative to PBS control.

Table 19

Dose-dependent inhibition of murine Factor 11 protein by ISIS 404071 in BALB/c mice

Dose in mg/kg	% Inhibition
5	39
10	67
25	89
50	96

Example 11: *In vivo* effect of antisense inhibition of murine Factor 11 in the $FeCl_3$ induced venous thrombosis (VT) model as compared to warfarin

Treatment

ISIS 404071 and warfarin (COUMADIN) were evaluated in the $FeCl_3$ induced VT mouse model. Six groups of BALB/c mice were treated with 1.25 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks.

Two days after receiving the last dose of ISIS 404071, mice were anesthetized with 150 mg/kg ketamine mixed with 10 mg/kg xylazine administered by intraperitoneal injection. An additional 6 groups of BALB/c mice were treated with 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, and 5 mg/kg of warfarin, administered intraperioneally daily for 6 days. Four hours after the last dose of warfarin, mice were anesthetized with 150 mg/kg ketamine mixed with 10 mg/kg xylazine administered by intraperitoneal injection. Two control groups of BALB/c mice were treated with PBS, administered subcutaneously twice a week for 3 weeks. Two days after the last dose of PBS, mice in both groups were anesthetized with 150 mg/kg ketamine mixed with 10 mg/kg xylazine administered by intraperitoneal injection. Thrombus formation was induced with $FeCl_3$ in all groups of mice except the first control group.

In mice undergoing $FeCl_3$ treatment, thrombus formation was induced by applying a piece of filter paper (2 x 4 mm) pre-saturated with 10 % $FeCl_3$ solution directly on the vena cava. After 3 minutes of exposure, the filter paper was removed. Thirty minutes after the filter paper application, a fixed length of the vein containing the thrombus was dissected out for platelet analysis. Liver was collected for RNA analysis.

RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of Factor 11. Results are presented as percent inhibition of Factor 11, relative to PBS control. As shown in Table 20, treatment with ISIS 404071 resulted in significant dose-dependent reduction of Factor 11 mRNA in comparison to the PBS control. Conversely, treatment with warfarin did not result in significant reduction of Factor 11 as compared to the PBS control.

Table 20

Dose-dependent reduction of Factor 11 mRNA in the $FeCl_3$ induced venous thrombosis model

Treatment	Dose in mg/kg	% inhibition
Warfarin	0.5	0
	1	0
	2	1
	3	5
	4	8
	5	11
ISIS 404071	1.25	0
	2.5	8

	5	62
	10	78
	20	92
	40	96

Quantification of Platelet Composition

Real-time PCR quantification of platelet factor-4 (PF-4) was used to quantify platelets in the vena cava as a measure of thrombus formation. Results are presented as a percentage of PF-4 in ISIS 404071 or warfarin treated mice, as compared to the two PBS-treated control groups. As shown in Table 21, treatment with ISIS 404071 resulted in a dose-dependent reduction of PF-4 in comparison to the PBS control for dosages of 5 mg/kg and higher. Treatment with warfarin resulted in a reduction of PF-4 in comparison to the PBS control for dosages of 2 mg/kg and higher. Therefore, reduction of Factor 11 by the compounds provided herein is useful for inhibiting thrombus and clot formation.

Table 21

Analysis of thrombus formation by real-time PCR quantification of PF-4 in the $FeCl_3$ induced venous thrombosis model

	Dose in mg/kg	PF-4
PBS-$FeCl_3$		0
PBS+$FeCl_3$		100
Warfarin	0.5	128
	1	124
	2	80
	3	21
	4	12
	5	33
ISIS 404071	1.25	143
	2.5	120
	5	95
	10	21
	20	37
	40	20

Example 12: *In vivo* effect of antisense inhibition of murine Factor 11 compared to warfarin in a tail bleeding assay

Treatment

Tail-bleeding was measured to observe whether treatment with ISIS 404071 or warfarin causes internal hemorrhage in mice. ISIS 404071 and warfarin (COUMADIN) were evaluated in the tail bleeding assay. Six groups of BALB/c mice were treated with 1.25 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks. An additional 6 groups of BALB/c mice were treated with 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, and 5 mg/kg of warfarin, administered intraperioneally daily for 6 days. A separate control group of BALB/c mice was treated with PBS, administered subcutaneously twice a week for 3 weeks.

Tail-bleeding Assay

Two days after the final treatment of ISIS 404071, warfarin, or PBS, mice were placed in a tail bleeding chamber. Mice were anesthetized in the chamber with isoflurane and a small piece of tail (approximately 4mm from the tip) was cut with sterile scissors. The tail cut was immediately placed in a 15 mL Falcon tube filled with approximately 10 mL of 0.9% NaCl buffer solution warmed to 37°C. The blood was collected over the course of 40 minutes. The saline filled tubes were weighed both before and after bleeding. The results are provided in Table 22.

Treatment with ISIS 404071 did not affect bleeding as compared to PBS treated mice. However, warfarin did increase bleeding in mice as compared to the PBS control. Increased doses of warfarin correlated positively with increased blood loss. These data suggest that the hemorrhagic potential of the compounds provided herein is low, especially in comparison to warfarin. These data taken with the results provided in example 11 suggest inhibition of Factor 11 with the compounds described herein are useful for providing antithrombotic activity without associated bleeding risk.

Table 22

Tail bleeding assay in the $FeCl_3$ induced venous thrombosis model

Treatment	Dose in mg/kg	Blood (g)
PBS	0	0.01
Warfarin	0.5	0.07
	1	0.35

	2	0.39
	3	0.51
	4	0.52
	5	0.76
ISIS 404071	1.25	0.00
	2.5	0.00
	5	0.03
	10	0.00
	20	0.06
	40	0.03

Example 13: *In vivo* effect of antisense inhibition of murine Factor 11 compared to warfarin on PT and aPTT

Treatment

PT and aPTT were measured using PPP from mice treated with ISIS 404071 or warfarin. Six groups of BALB/c mice were treated with 1.25 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks. An additional 6 groups of BALB/c mice were treated with 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, and 5 mg/kg of warfarin, administered intraperioneally daily for 6 days. In a control group, BALB/c mice were treated with PBS, administered subcutaneously mice twice a week for 3 weeks. Two days after the final dose was administered, PPP was collected and PT and aPTT assays were performed.

PT and aPTT assay

PT and aPTT values provided in Table 16 are reported as International Normalized Ratio (INR) values. INR values for PT and aPTT were determined by dividing the PT or aPTT value for each experimental group (i.e. 5 mg/kg, 10 mg/kg, 25 mg/kg, and 50 mg/kg treatment with ISIS 404071) by the PT or aPTT for the PBS treated group. This ratio was then raised to the power of the International Sensitivity Index (ISI) of the tissue factor used. As shown in Table 23, PT in warfarin treated mice is significantly prolonged at every dosage. aPTT in warfarin treated mice was prolonged, particularly at dosages of 1 mg/kg and higher. ISIS 404071 did not significantly affect PT, but did prolong aPTT; however, not as significantly as in warfarin treated mice. These data suggest that ISIS 404071 affects the contact activation pathway, but not the extrinsic pathway of blood coagulation whereas warfarin affects both the contact activation pathway and the extrinsic pathway of blood coagulation.

Table 23

Effect of ISIS 404071 and warfarin on PT and aPTT in BALB/c mice

Treatment	Dose in mg/kg	PT INR	aPTT INR
Warfarin	0.5	1.41	1.10
	1	2.03	1.31
	2	2.77	1.54
	3	22.76	2.90
	4	6.74	2.18
	5	9.20	2.29
ISIS 404071	1.25	0.99	0.98
	2.5	1.01	1.03
	5	1.07	1.09
	10	1.08	1.29
	20	1.09	1.32
	40	0.98	1.64

Example 14: *In vivo* effect of antisense inhibition of murine Factor 11 in the $FeCl_3$ induced venous thrombosis (VT) model as compared to Apixaban

Treatment

ISIS 404071 and Apixaban were evaluated in the $FeCl_3$ induced VT mouse model. Six groups of BALB/c mice were treated with 1.25 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks. Two days after receiving the last dose of ISIS 404071, mice were anesthetized with 150 mg/kg ketamine mixed with 10 mg/kg xylazine administered by intraperitoneal injection. An additional 6 groups of BALB/c mice were treated with 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, and 5 mg/kg of Apixaban, administered subcutaneously one time. Twenty minutes after receiving Apixaban, mice were anesthetized with 150 mg/kg ketamine mixed with 10 mg/kg xylazine administered by intraperitoneal injection. Two control groups of BALB/c mice were treated with PBS, administered subcutaneously twice a week for 3 weeks. Two days after the last dose of PBS, mice in both groups were anesthetized with 150 mg/kg ketamine mixed with 10 mg/kg xylazine administered by intraperitoneal injection. Thrombus formation was induced with $FeCl_3$ in all of the mice except the first control group.

In mice undergoing $FeCl_3$ treatment, thrombus formation was induced by applying a piece of filter paper (2 x 4 mm) pre-saturated with 10 % $FeCl_3$ solution directly on the vena cava. After 3 minutes of exposure, the filter paper was removed. Thirty minutes after the filter paper application, a fixed length of the vein containing the thrombus was dissected out for platelet analysis. Liver was collected for RNA analysis.

RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of Factor 11. Results are presented as percent inhibition of Factor 11, relative to PBS control. As shown in Table 24, treatment with ISIS 404071 resulted in significant dose-dependent reduction of Factor 11 mRNA in comparison to the PBS control. Conversely, treatment with Apixaban did not result in significant reduction of Factor 11 as compared to the PBS control.

Table 24

Dose-dependent reduction of Factor 11 mRNA in the $FeCl_3$ induced venous thrombosis model

	Dose in mg/kg	% inhibition
Apixaban	0.5	5
	2	8
	5	12
	10	2
	20	0
ISIS 404071	1.25	15
	2.5	44
	5	63
	10	76
	25	91
	50	95

Quantification of Platelet Composition

Real-time PCR quantification of platelet factor-4 (PF-4) was used to quantify platelets in the vena cava as a measure of thrombus formation. As shown in Table 25, treatment with ISIS 404071 resulted in reduction of PF-4 in comparison to the PBS control. Treatment with Apixaban also resulted in reduction of PF-4, in comparison to the PBS control. Results are presented as a percentage of PF-4 in ISIS 404071 or Apixaban treated mice, as compared to the two PBS-treated control groups.

Table 25

Analysis of thrombus formation by real-time PCR quantification of PF-4 in the $FeCl_3$ induced venous thrombosis model

Treatment	Dose in mg/kg	PF-4
PBS-$FeCl_3$		0
PBS+$FeCl_3$		100
Apixaban	0.5	67
	2	46
	5	15
	10	5
	20	26
ISIS 404071	1.25	42
	2.5	87
	5	60
	10	28
	25	14
	50	4

Example 15: *In vivo* effect of antisense inhibition of murine Factor 11 compared to Apixaban in the tail bleeding assay

Treatment

Tail bleeding was measured to observe whether treatment with ISIS 404071 or warfarin causes internal hemorrhage in mice. ISIS 404071 and Apixaban were evaluated in the tail bleeding model. Six groups of BALB/c mice were treated with 1.25 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks. An additional 6 groups of BALB/c mice were treated with 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, and 5 mg/kg of Apixaban, administered in a single subcutaneous dose. A separate control group of BALB/c mice was treated with PBS, administered subcutaneously twice a week for 3 weeks.

Tail-bleeding Assay

Two days after the final treatment of ISIS 404071, Apixaban, or PBS, mice were placed in a tail bleeding chamber. Mice were anesthetized in the chamber and a small piece of tail (approximately 4 mm from the tip) was cut with sterile scissors. The cut tail was immediately placed in a 15 mL Falcon tube filled with approximately 10 mL of 0.9% NaCl buffer solution warmed to 37^{0}C. The blood was collected over the course of 40 minutes. The saline filled tubes were weighed before and after bleeding.

As shown in Table 26, treatment with ISIS 404071 did not affect bleeding as compared to PBS treated mice. However, Apixaban did increase bleeding in mice as compared to the PBS control. Increased doses of Apixaban correlated positively with increased blood loss. These data suggest that the hemorrhagic potential of the compounds provided herein is low, especially in comparison to Apixaban. These data taken with the results provided in example 14 suggest inhibition of Factor 11 with the compounds described herein are useful for providing antithrombotic without associated bleeding risk.

Table 26
Tail bleeding assay in BABL/c mice

	mg/kg	Blood (g)
PBS	0	0.06
Apixaban	0.5	0.03
	2	0.34
	5	0.37
	10	0.40
	20	0.52
ISIS 404071	1.25	0.00
	2.5	0.03
	5	0.00
	10	0.04
	25	0.01
	50	0.01

Example 16: ***Ex vivo*** **effect of antisense inhibition of murine Factor 11 in combination with LOVENOX**

Treatment

Three groups of BALB/c mice were treated with 10 mg/kg, 20 mg/kg, or 40 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks. A control mouse group was treated with PBS, administered twice a week for 3 weeks. Five days after the last dose, the mice were sacrificed and plasma was collected. The low-molecular-weight (LMW) heparin, LOVENOX, was administered to the plasma *ex vivo* at varying concentrations of 0μg/ml, 2.5 μg/ml, 5.0μg/ml, and 7.5μg/ml. PT and aPTT were measured 20 minutes after LOVENOX was administered.

PT and aPTT Assay

As shown in Table 27, treatment with LOVENOX increases PT in a dose-dependent manner. Treatment with ISIS 404071 does not significantly increase PT. PT is not significantly affected by treatment with ISIS 404071. There is no evidence of a combinational effect on PT in ISIS 404071 and LOVENOX treated plasma.

Table 27

Effect of combination of ISIS 404071 and LOVENOX on PT INR in murine plasma

ISIS 404071 (mg/kg)	LOVENOX (mg/ml)			
	0	**2.5**	**5.0**	**7.5**
0	1.00	1.02	1.10	1.12
10	0.97	1.07	1.10	1.12
20	1.00	1.10	1.07	1.10
40	0.97	1.02	1.07	1.10

As shown in Table 28, treatment with LOVENOX increases aPTT in a dose-dependent manner. Treatment with ISIS 404071 also increases aPTT in a dose-dependent manner. Furthermore, the combined treatment of ISIS 404071 and LOVENOX appears to have a synergistic effect on aPTT.

Table 28

Effect of combination of ISIS 404071 and LOVENOX on aPTT INR in murine plasma

ISIS 404071 mg/kg	LOVENOX (mg/ml)			
	0	**2.5**	**5.0**	**7.5**
0	1.00	1.53	2.10	2.70
10	1.14	1.76	2.39	3.20
20	1.28	1.95	2.83	3.65
40	1.52	2.66	n.d.	4.78

n.d.= no data

Example 17: *In vivo* effect of antisense inhibition of murine Factor 11 in combination with LOVENOX in the $FeCl_3$ induced venous thrombosis (VT) model

Treatment

The combination of ISIS 404071 and LOVENOX were evaluated in the $FeCl_3$ induced VT mouse model. Four groups of BALB/c mice were treated with 15 mg/kg, 30 mg/kg, 45 mg/kg, or 60 mg/kg of LOVENOX, administered subcutaneously once daily for 3 days. An additional 4 groups of BALB/c mice were treated with 20 mg/kg of ISIS 404071, administered subcutaneously twice weekly for 3 weeks. After the last dose of ISIS 404071, mice were treated with 15 mg/kg, 30 mg/kg, 45 mg/kg, or 60 mg/kg of LOVENOX, administered subcutaneously once daily for 3 days. Two control groups of BALB/c mice were treated with PBS, administered subcutaneously twice a week for 3 weeks. Thrombus formation was induced with $FeCl_3$ in all of the mice except the first control group. All mice were anesthetized with 150 mg/kg of ketamine mixed with 10 mg/kg of xylazine administered by intraperitoneal injection.

In mice undergoing $FeCl_3$ treatment, thrombus formation was induced by applying a piece of filter paper (2 x 4 mm) pre-saturated with 10 % $FeCl_3$ solution directly on the vena cava. After 3 minutes of exposure, the filter paper was removed. Thirty minutes after the filter paper application, a fixed length of the vein containing the thrombus was dissected out for platelet analysis.

Quantification of Platelet Composition

Real-time PCR quantification of PF-4 was used to quantify platelets in the vena cava as a measure of thrombus formation. As shown in Table 29, treatment with LOVENOX resulted in a reduction of PF-4 in comparison to the PBS control. Treatment with LOVENOX in combination with ISIS 404071 resulted in a higher reduction of PF-4 in comparison to LOVENOX alone.

Table 29

Analysis of thrombus formation by real-time PCR quantification of PF-4 in the $FeCl_3$ induced venous thrombosis model

Treatment	mg/kg	PF-4
PBS-$FeCl_3$		0
PBS+$FeCl_3$		100
LOVENOX	15	57
	30	33
	45	10
	60	5
LOVENOX (+ ISIS 404071)	15	0
	30	0
	45	11
	60	5

Example 18: *In vivo* effect of antisense inhibition of murine Factor 11 in combination with LOVENOX on bleeding

Treatment

Tail-bleeding was measured to observe whether treatment with ISIS 404071 and LOVENOX causes internal hemorrhage in mice. ISIS 404071 was administered subcutaneously at a dosage of 20 mg/kg twice a week for 3 weeks to 4 groups of BALB/c mice, and LOVENOX was administered subcutaneously at varying dosages of 15 mg/kg, 30 mg/kg, 45 mg/kg, and 60 mg/kg once daily on the last three days of ISIS 404071 treatment. In a fifth group, ISIS 404071 was administered subcutaneously to BALB/c mice at a dosage of 20 mg/kg twice a week for 3 weeks. In a sixth group, PBS was administered subcutaneously twice a week for three weeks to BALB/c mice, as a control.

Tail-bleeding Assay

Two days after receiving their final treatment, mice were placed in a tail bleeding chamber. Mice were anesthetized in the chamber with isoflurane and a small piece of tail (approximately 4 mm from the tip) was cut with sterile scissors. The cut tail was immediately placed in a 15 mL Falcon tube filled with approximately 10 mL of 0.9% NaCl buffer solution warmed to 37 °C. The blood was collected over the course of 40 minutes. The saline filled tubes were weighed both before and after bleeding.

As shown in Table 30, LOVENOX increased bleeding in mice compared to the PBS treated mice. Increased doses of LOVENOX correlated positively with increased blood loss. ISIS 404071combined with LOVENOX did not significant increase bleeding beyond the increased blood loss shown in LOVENOX only treated mice.

Table 30

Tail bleeding assay comparing LOVENOX and the combination of LOVENOX and ISIS 404071

	Dose in mg/kg	Blood (g)
PBS		0.05
LOVENOX	15	0.11
	30	0.20
	45	0.27
	60	0.47
LOVENOX (+ISIS 404071)	15	0.14
	30	0.19
	45	0.36
	60	0.61

Example 19: ***In vivo*** **effect of antisense inhibition of murine Factor 11 in combination with LOVENOX on PT and aPTT**

Treatment

PT and aPTT were measured using PPP from mice treated with ISIS 404071 in combination with LOVENOX. In the first cohort, ISIS 404071 was administered subcutaneously to BALB/c mice at a dosage of 25 mg/kg twice a week for 3 weeks. Plasma was collected from these mice 5 days after receiving the last dose of ISIS 404071. In the second cohort, LOVENOX was administered subcutaneously to BALB/c mice at a dosage of 20 mg/kg once daily for three days. Plasma was collected from these mice 4 hours after receiving the last dose of LOVENOX. In the third cohort, ISIS 404071 was administered subcutaneously to BALB/c mice at a dosage of 20 mg/kg twice a week for 3 weeks, and 2 days after receiving the last dose of ISIS 404071, LOVENOX was administered subcutaneously at a dosage of 20 mg/kg once daily. Plasma was collected from these mice 4 hours after the last dose of LOVENOX. In a fourth cohort, PBS was administered subcutaneously twice a week for three weeks, as a control. Plasma was collected from these mice 5 days after the last dose.

PT and aPTT assay

PT and aPTT values provided in Table 31 are reported as International Normalized Ratio (INR) values. As shown in Table 31, PT is not significantly affected by treatment with ISIS 404071, LOVENOX, or treatment with ISIS 40471 combined with LOVENOX. These data suggest that there is no combinational effect on PT by ISIS 404071 combined with LOVENOX. Also shown in Table 31, treatment with LOVENOX and treatment with ISIS 404071 combined with LOVENOX increase aPTT. These data suggest that the combined treatment of ISIS 404071 and LOVENOX has an additive effect on aPTT.

Table 31

Effect of combination of ISIS 404071 and LOVENOX on PT and aPTT in murine plasma

	PT INR	aPTT INR
ISIS 404071	0.95	1.31
LOVENOX	1.04	2.04
404071+LOVENOX	1.04	2.58

Example 20: *In vivo* effect of antisense inhibition of murine Factor 11 in combination with Apixaban on PT and aPTT

Treatment

PT and aPTT were measured using PPP from mice treated with ISIS 404071 in combination with Apixaban. In the first cohort, ISIS 404071 was administered subcutaneously to BALB/c mice at a dosage of 25 mg/kg twice a week for 3 weeks. Plasma was collected from these mice 5 days after receiving the last dose of ISIS 404071. In the second cohort, Apixaban was administered subcutaneously to BALB/c mice at a dosage of 6 mg/kg twice daily for three days. Plasma was collected from these mice 20 minutes after receiving the last dose of Apixaban. In the third cohort, ISIS 404071 was administered subcutaneously to BALB/c mice at a dosage of 20 mg/kg twice a week for 3 weeks, and Apixaban was administered subcutaneously at a dosage of 6 mg/kg twice daily on the last three days of ISIS 404071 treatment. Plasma was collected from these mice 20 minutes after receiving the last dose of Apixaban. In a fourth cohort, PBS was administered subcutaneously twice a week for three weeks, as a control. Plasma was collected 5 days after the last dose of PBS.

PT and aPTT assay

PT and aPTT values provided in Table 32 are reported as International Normalized Ratio (INR) values. As shown in Table 32, PT is not significantly affected by treatment with ISIS 404071. However, Apixaban and Apixaban combined with ISIS 404071 increased PT. Also shown in Table 32, Apixaban, ISIS 404071, and ISIS 404071 combined with Apixaban increase aPTT.

Table 32

Effect of combination of ISIS 404071 and Apixaban on PT and aPTT in murine plasma

	PT INR	aPTT INR
ISIS 404071	0.95	1.31
Apixaban	3.25	1.44
404071+Apixaban	3.50	2.26

Example 21: *In vivo* effect of antisense inhibition of murine Factor 11 in combination with warfarin on PT and aPTT

Treatment

PT and aPTT were measured using PPP from mice treated with ISIS 404071 in combination with warfarin. Two groups of BALB/c mice were treated with either 25 mg/kg or 50 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks. Plasma was collected from each group 5 days after the last dose was administered. In a third group, BALB/c mice were treated with 2 mg/kg of warfarin once daily for 5 days. Plasma was collected 6 hours after the last dose of warfarin was administered. Two additional groups of BALB/c mice were treated with either 25 mg/kg or 50 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks and warfarin was administered subcutaneously at a dosage of 2 mg/kg once daily on the last 5 days of ISIS 404071 treatment. Plasma was collected from each group 6 hours after the last warfarin treatment. In a final group of BALB/c mice, PBS was administered subcutaneously twice a week for three weeks, as a control. Plasma was collected 5 days after the last PBS treatment.

PT and aPTT Assay

PT and aPTT values provided in Table 33 are reported as International Normalized Ratio (INR) values. As shown in Table 33, PT is not affected by treatment with PBS or ISIS 404071 at either dosage. However, treatment with 2 mg/kg warfarin, 25 mg/kg ISIS 404071 in combination with 2 mg/kg warfarin, and 50 mg/kg ISIS 404071 in combination with 2 mg/kg warfarin increase PT. These data suggest that the combined treatment of ISIS 404071 and warfarin has an additive effect on PT. Also shown in Table 33, aPTT is affected by treatment with ISIS 404071 and warfarin. The combination of ISIS 404071 and warfarin show an increase in aPTT greater than either drug alone. These data suggest that the combined treatment of ISIS 404071 and warfarin has a synergistic effect on aPTT.

Table 33

Effect of combination of ISIS 404071 and warfarin on PT and aPTT in murine plasma

	Dose in mg/kg	PT INR	aPTT INR
ISIS 404071	25	0.98	1.37
	50	0.93	1.49
Warfarin	2	21.33	2.52

ISIS 404071(+Warfarin)	25	25.77	4.45
	50	36.33	4.75

Example 22: *In vivo* antithrombotic effect of antisense inhibition of murine Factor 11 on mesenteric vein thrombosis in mice

Treatment

In a first cohort, ISIS 404071 was administered subcutaneously to C57BL/6 mice twice a week for three weeks at a dose of 50 mg/kg. In a second cohort, a control oligonucleotide, ISIS 405277 (AAGGACCTACACTATGGAAT; antisense oligonucleotide for Factor 2), incorporated herein as SEQ ID NO: 12 was administered subcutaneously to C57Bl/6 mice twice a week for three weeks at a dose of 50 mg/kg.

Platelet Preparation

Blood was collected from the retro-orbital venous plexus of naïve C57BL/6 mice by puncture and collected in polypropylene tubes containing 300 µl of heparin (30U/ml). Platelet rich plasma (PRP) was obtained by centrifugation at 1000 rpm for 5 min. The PRP was transferred to fresh tubes containing 2 µl of Prostaglandin I_2 (PGI_2) (2 µg/ml) and incubated at 37°C for 5 min. After centrifugation at 2600 rpm, pellets were resuspended in 1 ml modified Tyrode's-HEPES buffer (137 mM NaCl, 0.3 mM Na_2HPO_4, 2 mM KCl, 12 mM $NaHCO_3$, 5 mM HEPES, 5 mM glucose, 0.35% BSA, pH 7.2) containing 2 µl of PGI_2 and incubated at 37°C for 5 min. The suspended pellet was centrifuged at 2600 rpm for 5 min. To remove PGI_2, the washing step was repeated twice and platelets were fluorescently labeled with calcein AM 2.5 µg/mL (Molecular Probes, Eugene, OR) for 10 min at room temperature.

Intravital Microscopy for Thrombosis

Fluorescently-labeled platelets were injected intravenously in ISIS 404071 treated and control oligonucleotide treated C57BL/6 mice. The mice were anaesthetized with 2.5% avertin, and an incision was made through the abdominal wall to expose mesenteric veins 250-300-µm in diameter and having a shear rate of approximately 150 s^{-1}. The exposed mesentery was kept moist throughout the experiment by periodic superfusion with warmed (37°C) PBS. The mesentery was transluminated with a 12V, 100W, DC stabilized source. Veins were visualized using a Zeiss (Germany) Axiovert 135 inverted microscope (Objective 32X) connected to an SVHS video recorder (AG-6730; Panasonic, Tokyo, Japan) using a CCD video camera (Hamamatsu Photonic Systems, Hamamatsu City, Japan). Centerline erythrocyte velocity (V_{rbc}) was measured using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M College of Medicine, College Station, TX). Venular shear rate (τ) was calculated based on Poiseuille's Law for a newtonian fluid, $\tau = 8(V_{mean}/D_v)$, where D_v is the diameter of the venule and V_{mean} is estimated from the measured V_{rbc} using the empirical correlation $V_{mean} = V_{rbc}/1.6$.

Results Analysis

Mesenteric vein thrombosis was performed two days after the last antisense oligonucleotide injection. Thrombosis was induced by applying Whatman paper soaked in a 10 % $FeCl_3$ solution for 5 minutes on the mesenteric vein. The vein was monitored for 40 minutes, or until occlusion. The elapsed time before the first thrombus 30-50 μm in diameter and the elapsed time before blood stopped flowing for 30 seconds were observed.

Thrombus formation (30 μm in diameter) occurred in mice treated with ISIS 404071 at 14.8 ± 1.7 minutes. Thrombus formation (30 μm in diameter) occurred in control mice at 8.9 ± 0.6 minutes. Occlusive thrombi formed in control mice at 19.3 ± 0.8 min and all injured venules occluded. In contrast, the majority of the veins in ISIS 404071 treated mice did not occlude when observation was terminated 40 minutes after injury and those veins showing occlusion. The only vein showing occlusion in the ISIS 404071 treated mice occluded at 29.5 minutes and reopened after 5 minutes, prior to the end of the study.

Example 23: *In vivo* sense-oligonucleotide-antidote for antisense inhibition of murine Factor 11 in BALB/c mice

Treatment

The effect of the specific sense oligonucleotide to ISIS 404071 as an antidote was tested in BALB/c mice. In a first cohort, ISIS 404071 was administered subcutaneously to BALB/c mice twice a week for three weeks at a dose of 40 mg/kg. In a second cohort, ISIS 404057 was administered subcutaneously to BALB/c mice twice a week for three weeks at a dose of 40 mg/kg. The ISIS 404071 specific antidote, ISIS 418026 (CCTCTGAAAGTGGATTACCA; complementary to ISIS 404071), incorporated herein as SEQ ID NO: 13, was administered to both cohorts subcutaneously in a single injection of 90 mg/kg 48 hours after the final treatment of ISIS 404071 or

404057. In a third cohort, ISIS 404071 was administered subcutaneously to BALB/c mice twice a week for three weeks at a dose of 40 mg/kg. Following the last treatment of ISIS 404071, mice were injected subcutaneously injected with PBS. In a fourth cohort, ISIS 404057 was administered subcutaneously to BALB/c mice twice a week for three weeks at a dose of 40 mg/kg. Following the last treatment of ISIS 404057, mice were injected subcutaneously injected with PBS. Following antidote administration, a set of 4 mice from each cohort were sacrificed at 12 hours, 1 day, 2 days, 3 days, 7 days, and 14 days. Whole liver was collected for RNA analysis and PPP was collected for aPTT analysis.

RNA analysis

RNA was extracted from liver tissue for real-time PCR analysis of Factor 11. Results are presented as percent inhibition of Factor 11, relative to PBS control. As shown in Table 34, mice treated with ISIS 404071 without antidote showed progressive decrease in inhibition over the 14 day observation period. However, mice treated with ISIS 404071 and antidote showed an accelerated decrease in inhibition over the 14 day observation period in comparison to mice which did not receive antidote. Also shown in Table 34, treatment with ISIS 418026 had no effect on inhibition of Factor 11 mRNA expression in ISIS 404057 treated mice.

Table 34

Percent inhibition of mouse Factor 11 mRNA compared to PBS control

	12 hours	1 day	2 days	3 days	7 days	14 days
ISIS 404071	93	90	89	88	81	67
ISIS 404071 + ISIS 418026	90	87	72	66	57	31
ISIS 404057	n.d.	n.d.	n.d.	95	n.d.	n.d.
ISIS 404057 + ISIS 418026	n.d.	n.d.	n.d.	97	n.d.	n.d.

n.d.= no data

aPTT Assay

As shown in Table 35, mice treated with ISIS 404071 and antidote (ISIS 418026) showed progressive decrease of aPTT over the 14 day observation period compared to mice treated with ISIS 404071 without antidote.

Table 35

Effect of antidote treatment on aPTT INR

	12 hours	1 day	2 day	3 day	7 day	14 day
ISIS 404071	1.51	1.30	1.35	1.27	1.18	1.05
ISIS 404071 + ISIS 418026	1.45	1.23	1.16	1.15	1.10	0.95

Example 24: *In vivo* Factor 7a protein-antidote for antisense inhibition of murine Factor 11 in BALB/c mice

Treatment

The effect of human Factor 7a (Factor VIIa) protein as an antidote for ISIS 404071 was tested in BALB/c mice. Two experimental groups of BALB/c mice were treated with 20 mg/kg of ISIS 404071, administered subcutaneously twice a week for 3 weeks. Two control groups of BALB/c mice were treated with PBS, administered subcutaneously twice a week for 3 weeks. Thrombus formation was induced with $FeCl_3$ in all of the mice except the first control group. Fifteen minutes before $FeCl_3$ treatment, the first experimental group was treated with 5 μg/kg of human Factor 7a protein antidote (product no. 407act, American Diagnostica Inc.). Two days after their last dose, all mice were anesthetized with 150 mg/kg of ketamine mixed with 10 mg/kg of xylazine administered by intraperitoneal injection.

In mice undergoing $FeCl_3$ treatment, thrombus formation was induced by applying a piece of filter paper (2 x 4 mm) pre-saturated with 10 % $FeCl_3$ solution directly on the vena cava. After 3 minutes of exposure, the filter paper was removed. Thirty minutes after the filter paper application, a fixed length of the vein containing the thrombus was dissected out for platelet analysis.

Quantification of Platelet Composition

Real-time PCR quantification of platelet factor-4 (PF-4) was used to quantify platelets in the vena cava as a measure of thrombus formation. Results are presented as a percentage of PF-4 in antidote treated and untreated mice, as compared to the two PBS-treated control groups. As shown in Table 36, animals treated with human Factor 7a protein antidote expressed more PF-4 in comparison to animals treated with ISIS 404071 alone. These data indicate that human Factor 7a is successful in rescuing the effect of antisense oligonucleotide inhibition.

Table 36

Analysis of thrombus formation by real-time PCR quantification of PF-4 in the $FeCl_3$ induced venous thrombosis model

Treatment	PF-4
PBS-$FeCl_3$	0
PBS+$FeCl_3$	100
ISIS 404071	18
ISIS 404071+hFV7a	68

Example 25: *In vivo* antisense inhibition of murine Factor 11 in the collagenase-induced intracerebral hemorrhage model

Treatment

ISIS 404071 and warfarin (COUMADIN) were examined in the collegenase-induced intracerebral hemorrhage model. In a first cohort, ISIS 404071 was administered subcutaneously to BALB/c mice twice a week for two weeks at a dose 40 mg/kg. In a second cohort, warfarin was administered intraperioneally to mice twice a week for two weeks at a dose of 2 mg/kg. In a third cohort, ISIS 421208 (TCGGAAGCGACTCTTATATG, 8 mismatches to murine Factor 11, incorporated herein as SEQ ID NO: 14) was administered subcutaneously to BALB/c mice twice a week for two weeks at a dose 40 mg/kg. In a fourth cohort, PBS was administered to BALB/c mice twice a week for two weeks.

Two days after receiving their final dose, all mice in all cohorts were anesthetized with 5 µg/g of avertin. Next, the mice were injected at -1 mm AP, 1 mm R ML, -4 mm DV from bregma flat skull with a 10 µL Hamilton syringe containing 0.075 U collagenase (150 U/mL). Collagenase was delivered over 5 minutes and the needle was kept in place for an additional 5 minutes to prevent reflux. The mice were then analyzed for hemorrhagic size, neurologic deficit score, and mortality.

Table 37 presents the hemorrhage volume detected in mice after collagenase treatment, Table 38 presents the neurologic deficit score of the mice, and Table 39 presents the mortality rate of the mice. Neurological deficit is measured by a standard scoring system where no deficiency is zero and severe deficit is five. Collectively, the data suggest that ISIS 404071 did not have a significant effect on the hemorrhagic size, neurologic deficit score, or mortality of the mice. Thus, risk of intracerebral hemorrhage (a risk factor for warfarin treated individuals) is significantly reduced in ISIS 404071 treated mice in comparison to warfarin treated mice.

Table 37

Hemorrhagic volume after collagenase treatment

	Volume (mm^3)
PBS	51
ISIS 421208	41
ISIS 404071	38

Table 38

Neurologic Deficit Score after collagenase treatment

	Score
PBS	2.4
ISIS 421208	2.0
ISIS 404071	3.8

Table 39

Mortality after collagenase treatment

	% mortality
PBS	0
ISIS 421208	0
ISIS 404071	20
Warfarin	80

Example 26: *In vivo* effect of antisense inhibition of murine Factor 11 in combination with PLAVIX in the $FeCl_3$ induced venous thrombosis (VT) model

Treatment

The combination of ISIS 404071 and PLAVIX was evaluated in the $FeCl_3$ induced VT mouse model. Four groups of eight BALB/c mice, weighing approximately 25 g each, were treated with 6.25 mg/kg, 12.50 mg/kg, 25.00 mg/kg, or 50.00 mg/kg of PLAVIX. Mice were given two doses of PLAVIX on day one and one dose of PLAVIX on day two, two hours before surgery.

An additional four groups of eight BALB/c mice, weighing approximately 25 g each, were treated with 20 mg/kg of ISIS 404071, administered subcutaneously twice a week for three weeks. After the last dose of ISIS 404071, mice were treated with 6.25 mg/kg, 12.50 mg/kg, 25.00 mg/kg, or 50.00 mg/kg of PLAVIX. Two doses of PLAVIX were administered to the mice on day one and one dose of PLAVIX was administered on day two, two hours before surgery.

Two control groups of eight BALB/c mice, weighing approximately 25 g each, were not treated with ISIS 404071 or PLAVIX. An additional two control groups of eight BALB/c mice, weighing approximately 25 g each, were treated with 20 mg/kg of ISIS 404071, administered subcutaneously twice a week for three weeks, but were not treated with PLAVIX. Thrombus formation was induced with $FeCl_3$ in all of the mice except the first and third control groups. All mice were anesthetized with 150 mg/kg of ketamine mixed with 10 mg/kg of xylazine administered by intraperitoneal injection.

In mice undergoing $FeCl_3$ treatment, thrombus formation was induced by applying a piece of filter paper (2 x 4 mm) pre-saturated with 10% $FeCl_3$ solution directly on the inferior vena cava. After 3 minutes of exposure, the filter paper was removed. Thirty minutes after the filter paper application, a fixed length of the vein containing the thrombus was dissected out for platelet analysis.

Quantification of Platelet Composition

Real-time PCR quantification of PF-4 was used to quantify platelets in the vena cava as a measure of thrombus formation. As shown in Table 40, treatment with PLAVIX resulted in a reduction of PF-4 in comparison to the PBS control. Treatment with PLAVIX in combination with ISIS 404071 resulted in a higher reduction of PF-4 in comparison to PLAVIX alone. Therefore, the combination of anti-platelet therapy with Factor 11 ASO increases antithrombotic activity. Data is presented as percent of PF-4 mRNA as compared to the PBS+ $FeCl_3$ control.

Table 40
Analysis of thrombus formation by real-time PCR quantification of PF-4 in the FeCl3 induced venous thrombosis model

Treatment	ISIS 404071 mg/kg	PLAVIX mg/kg	PF-4
PBS- $FeCl_3$	0	0	29
PBS+ $FeCl_3$	0	0	100
PLAVIX only	0	6.25	59
	0	12.50	37
	0	25.00	30
	0	50.00	30
ISIS 404071-$FeCl_3$	20	0	27
ISIS 404071+$FeCl_3$	20	0	40
PLAVIX (+ ISIS 404071)	20	6.25	35
	20	12.50	38
	20	25.00	25
	20	50.00	35

Example 27: *In vivo* effect of antisense inhibition of murine Factor 11 in combination with PLAVIX on bleeding

Treatment

Tail-bleeding was measured to observe whether treatment with ISIS 404071 in combination with PLAVIX causes an increase in bleeding tendency. ISIS 404071 was administered subcutaneously at a dosage of 20 mg/kg twice a week for 3 weeks to 5 groups of eight BALB/c mice. After the last dose of ISIS 404071, mice were treated with 0 mg/kg, 6.25 mg/kg, 12.50 mg/kg, 25.00 mg/kg, or 50.00 mg/kg of PLAVIX. Two doses of PLAVIX were administered to the mice on day one and one dose of PLAVIX was administered on day two, two hours before bleeding.

An additional 5 groups of eight BABL/c mice were treated similarly, except they did not receive ISIS 404071 injections.

Tail-Bleeding Assay

Two hours after receiving their final treatment, mice were placed in a tail bleeding chamber. Mice were anesthetized in the chamber with isoflurane and a small piece of tail (approximately 4 mm from the tip) was cut with sterile scissors. The cut tail was immediately placed in a 15 mL

Falcon tube filled with approximately 10 mL of 0.9% NaCl buffer solution warmed to 37°C. The blood was collected for the course of 40 minutes. The saline filled tubes were weighed both before and after bleeding.

Taken with the results of Example 26, these data show that the combination of anti-platelet therapy with Factor 11 ASO increases antithrombotic activity without increased bleeding risk.

Table 41

Tail bleeding assay comparing PLAVIX and the combination of PLAVIX and ISIS 404071

Treatment	ISIS 404071 mg/kg	PLAVIX mg/kg	Blood (g)
No treatment	0	0	0.040
PLAVIX only	0	6.25 mg/kg	0.075
	0	12.50 mg/kg	0.205
	0	25.00 mg/kg	0.524
	0	50.00 mg/kg	0.628
ISIS 404071 only	20 mg/kg	0	0
PLAVIX (+ ISIS 404071)	20 mg/kg	6.25 mg/kg	0.065
	20 mg/kg	12.50 mg/kg	0.300
	20 mg/kg	25.00 mg/kg	0.401
	20 mg/kg	50.00 mg/kg	0.577

Example 28: *In vivo* effect of a Factor Xa small molecule inhibitor in combination with PLAVIX on bleeding

Treatment

Tail-bleeding was measured to observe whether treatment with a Factor 10a small molecule in combination with PLAVIX causes an increase in bleeding tendency. Five groups of eight BALB/c mice were treated with 0 mg/kg, 6.25 mg/kg, 12.50 mg/kg, 25.00 mg/kg, or 50.00 mg/kg of PLAVIX. Mice were given two doses of PLAVIX on day one and one dose of PLAVIX on day two, two hours before bleeding.

An additional five groups of eight BALB/c mice were treated with 0 mg/kg, 6.25 mg/kg, 12.50 mg/kg, 25.00 mg/kg, or 50.00 mg/kg of PLAVIX. Mice were given two doses of PLAVIX on day one and one dose of PLAVIX on day two, two hours before bleeding. These mice were also treated with 0.5 mg/kg of Apixaban, a small molecule Factor 10a inhibitor, intraperitoneally one time 20 minutes before bleeding.

Tail-Bleeding Assay

Two hours after receiving their final treatment, mice were placed in a tail bleeding chamber. Mice were anesthetized in the chamber with isoflurane and a small piece of tail (approximately 4 mm from the tip) was cut with sterile scissors. The cut tail was immediately placed in a 15 mL Falcon tube filled with approximately 10 mL of 0.9% NaCl buffer solution warmed to 37°C. The blood was collected for the course of 40 minutes. The saline filled tubes were weighed both before and after bleeding.

As shown below in Table 42, these data show that the combination of anti-platelet therapy with a small molecule Factor 10a inhibitor, such as Apixaban, increases bleeding risk. Therefore, treatment with the combination of anti-platelet therapy with a Factor 11 ASO provides a better safety profile in comparison to the safety profile of a combination of anti-platelet therapy with a small molecule Factor 10a inhibitor.

Table 42

Tail bleeding assay comparing PLAVIX, Apixaban, and the combination of PLAVIX and Apixaban

Treatment	Apixaban mg/kg	PLAVIX mg/kg	Blood (g)
No treatment	0	0	0.002
PLAVIX only	0	6.25 mg/kg	0.061
	0	12.50 mg/kg	0.149
	0	25.00 mg/kg	0.246
	0	50.00 mg/kg	0.258
Apixaban only	0.5 mg/kg	0	0.004
PLAVIX (+ Apixaban)	0.5 mg/kg	6.25 mg/kg	0.258
	0.5 mg/kg	12.50 mg/kg	0.252
	0.5 mg/kg	25.00 mg/kg	0.361
	0.5 mg/kg	50.00 mg/kg	0.363

Example 29: Time course of *in vivo*, antisense-mediated reduction of murine Factor 11 and corresponding anticoagulation in blood

Treatment

The time course of antisense-mediated reduction of murine Factor 11 mRNA was observed in BALB/c mice. One dose of 50 mg/kg ISIS 404071 was administered subcutaneously to BALB/c mice. Following ISIS 404071 administration, mice were sacrificed at 12 hours, 1 day, 2 days, 3 days, 4 days, 7 days, 14 days, 28 days, and 56 days. Whole liver was collected for RNA analysis and PPP was collected for aPTT analysis. A control group of mice was treated with one subcutaneous dose of PBS.

RNA analysis

RNA was extracted from liver tissue for real-time PCR analysis of Factor 11. Results are presented relative to PBS control. Mice treated with ISIS 404071 showed significant Factor 11 mRNA down-regulation by day 1. Mice began regaining Factor 11 mRNA expression by day 14. Mice regained full Factor 11 mRNA expression by day 28 and results from day 56 indicate that Factor mRNA was maintained at pre-treatment levels. Therefore, ISIS 404071 treated mice did not experience a rebound effect.

The rebound effect has been previously observed in antibody-mediated reduction of Factor 11 (Blood, First Edition Paper, prepublished online October 22, 2008; Prevention of vascular graft occlusion and thrombus-associated thrombin generation by inhibition of factor XI). Because over expression of Factor 11 can be damaging by leading to increased coagulation, these data suggest that antisense-mediated inhibition of Factor 11 is safer than antibody-mediated inhibition of Factor 11 since antisense-mediated inhibition of Factor 11 does not rebound.

aPTT Assay

aPTT values provided in Table 43 are reported as International Normalized Ratio (INR) values. INR values for aPTT were determined by dividing the aPTT value for ISIS 404071 treated mice by the aPTT for the PBS treated group. This ratio was then raised to the power of the International Sensitivity Index (ISI) of the tissue factor used. As shown in Table 43, mice treated with ISIS 404071 showed progressive decrease of aPTT until day 4 and then progressive increase to pre-treatment levels from day 7 to day 28.

Table 43

Effect of ISIS 404071 treatment on aPTT INR*

	12 hours	day 1	day 2	day 3	day 4	day 7	day 14	day 28	day 56
ISIS 404071	0	1.02	1.12	1.29	1.30	1.25	1.11	1.02	0

*values in Table 43 are approximate

Example 30: Antisense inhibition of human Factor 11 in HepG2 cells by oligonucleotides designed by microwalk

Additional gapmers were designed based on ISIS 416850 and ISIS 416858 (see Table 8 above). These gapmers were shifted slightly upstream and downstream (i.e. "microwalk") of ISIS 416850 and ISIS 416858. The microwalk gapmers were designed with either 5-8-5 MOE or 6-8-6 MOE motifs.

These microwalk gapmers were tested *in vitro*. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 8,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real-time PCR. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells.

ISIS 416850 and ISIS 416858, as well as selected gapmers from Tables 1 and 8 (i.e., ISIS 412206, ISIS 412223, ISIS 412224, ISIS 412225, ISIS 413481, ISIS 413482, ISIS 416825, ISIS 416848, ISIS 416849, ISIS 416850, ISIS 416851, ISIS 416852, ISIS 416853, ISIS 416854, ISIS 416855, ISIS 416856, ISIS 416857, ISIS 416858, ISIS 416859, ISIS 416860, ISIS 416861, ISIS 416862, ISIS 416863, ISIS 416864, ISIS 416865, ISIS 416866, and ISIS 416867) were retested *in vitro* along with the microwalk gapmers under the same condition as described above.

The chimeric antisense oligonucleotides in Table 44 were designed as 5-10-5 MOE, 5-8-5 and 6-8-6 MOE gapmers. The first two listed gapmers in Table 44 are the original gapmers (ISIS 416850 and ISIS 416858) from which ISIS 445493-445543 were designed via microwalk, and are designated by an asterisk. The 5-10-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleotides each. The 5-8-5 gapmers are 18 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleotides each. The 6-8-6 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising six nucleotides each. For each of the motifs (5-10-5, 5-8-5 and 6-8-6), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Human Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the human sequence. "Human Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted in the human sequence. Each gapmer listed in Table 44 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3). Each gapmer is Table 44 is also fully cross-reactive with the rhesus monkey Factor 11 gene sequence, designated herein as SEQ ID NO: 274 (exons 1-15 GENBANK Accession No. NW_001118167.1). 'Rhesus monkey start site' indicates the 5'-most nucleotide to which the gapmer is targeted in the rhesus monkey sequence. 'Rhesus monkey stop site' indicates the 3'-most nucleotide to which the gapmer is targeted to the rhesus monkey sequence.

As shown in Table 44, all of the microwalk designed gapmers targeted to the target region beginning at the target start site 1275 and ending at the target stop site 1317 (i.e. nucleobases 1275-1317) of SEQ ID NO: 1 exhibited at least 60% inhibition of Factor 11 mRNA. Similarly, all of the re-tested gapmers from Tables 1 and 8 exhibited at least 60 % inhibition.

Several of the gapmers exhibited at least 70% inhibition, including ISIS numbers: ISIS 412206, 412224, 412225, 413481, 413482, 416825, 416848, 416849, 416850, 416851, 416852, 416853, 416854, 416855, 416856, 416857, 416858, 416859, 416860, 416861, 416862, 416863, 416864, 416865, 416866, 416867, 445494, 445495, 445496, 445497, 445498, 445499, 445500, 445501, 445502, 445503, 445504, 445505, 445506, 445507, 445508, 445509, 445510, 445511, 445512, 445513, 445514, 445515, 445516, 445517, 445518, 445519, 445520, 445521, 445522, 445523, 445524, 445525, 445526, 445527, 445528, 445529, 445530, 445531, 445532, 445533, 445534, 445535, 445536, 445537, 455538, 445539, 445540, 445541, 445542, and 445543.

Several of the gapmers exhibited at least 80% inhibition, including ISIS numbers: ISIS 412206, 412224, 412225, 413481, 413482, 416825, 416848, 416849, 416850, 416851, 416852, 416853, 416854, 416855, 416856, 416857, 416858, 416859, 416860, 416861, 416862, 416863, 416864, 416865, 416866, 416867, 445494, 445495, 445496, 445497, 445498, 445500, 445501, 445502, 445503, 445504, 445505, 445506, 445507, 445508, 445509, 445510, 445513, 445514, 445519, 445520, 445521, 445522, 445525, 445526, 445529, 445530, 445531, 445532, 445533, 445534, 445535, 445536, 455538, 445541, and 445542.

Several of the gapmers exhibited at least 90% inhibition, including ISIS numbers: ISIS 412206, 416825, 416850, 416857, 416858, 416861, 445522, and 445531.

Table 44

Inhibition of human Factor 11 mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3)

ISIS No.	Human Start Site	Human Stop Site	Sequence (5' to 3')	Percent inhibition	Motif	SEQ ID No.	Rhesus monkey Start Site	Rhesus monkey Stop Site
*416850	1278	1297	TGCACAGTTT CTGGCAGGCC	91	5/10/2005	215	1277	1296
*416858	1288	1307	ACGGCATTGG TGCACAGTTT	90	5/10/2005	223	1287	1306
416825	680	699	GCCCTTCATGT CTAGGTCCA	90	5/10/2005	190	679	698
412206	738	757	CCGTGCATCTT TCTTGGCAT	91	5/10/2005	34	737	756
412223	1275	1294	ACAGTTTCTG GCAGGCCTCG	62	5/10/2005	51	1274	1293
445493	1275	1294	ACAGTTTCTG GCAGGCCTCG	69	6/8/2006	51	1274	1293
445518	1275	1292	AGTTTCTGGC AGGCCTCG	75	5/8/2005	242	1274	1291
416848	1276	1295	CACAGTTTCT GGCAGGCCTC	87	5/10/2005	213	1275	1294
445494	1276	1295	CACAGTTTCT GGCAGGCCTC	85	6/8/2006	213	1275	1294
445519	1276	1293	CAGTTTCTGG CAGGCCTC	81	5/8/2005	243	1275	1292
416849	1277	1296	GCACAGTTTC TGGCAGGCCT	88	5/10/2005	214	1276	1295
445495	1277	1296	GCACAGTTTC TGGCAGGCCT	89	6/8/2006	214	1276	1295
445520	1277	1294	ACAGTTTCTG GCAGGCCT	82	5/8/2005	244	1276	1293

445496	1278	1297	TGCACAGTTT CTGGCAGGCC	87	6/8/2006	215	1277	1296
445521	1278	1295	CACAGTTTCT GGCAGGCC	87	5/8/2005	245	1277	1294
416851	1279	1298	GTGCACAGTT TCTGGCAGGC	89	5/10/2005	216	1278	1297
445497	1279	1298	GTGCACAGTT TCTGGCAGGC	81	6/8/2006	216	1278	1297
445522	1279	1296	GCACAGTTTC TGGCAGGC	91	5/8/2005	246	1278	1295
413481	1280	1299	GGTGCACAGT TTCTGGCAGG	82	5/10/2005	114	1279	1298
445498	1280	1299	GGTGCACAGT TTCTGGCAGG	83	6/8/2006	114	1279	1298
445523	1280	1297	TGCACAGTTT CTGGCAGG	73	5/8/2005	267	1279	1296
416852	1281	1300	TGGTGCACAG TTTCTGGCAG	87	5/10/2005	217	1280	1299
445499	1281	1300	TGGTGCACAG TTTCTGGCAG	75	6/8/2006	217	1280	1299
445524	1281	1298	GTGCACAGTT TCTGGCAG	75	5/8/2005	247	1280	1297
416853	1282	1301	TTGGTGCACA GTTTCTGGCA	84	5/10/2005	218	1281	1300
445500	1282	1301	TTGGTGCACA GTTTCTGGCA	81	6/8/2006	218	1281	1300
445525	1282	1299	GGTGCACAGT TTCTGGCA	85	5/8/2005	248	1281	1298
416854	1283	1302	ATTGGTGCAC AGTTTCTGGC	86	5/10/2005	219	1282	1301
445501	1283	1302	ATTGGTGCAC AGTTTCTGGC	83	6/8/2006	219	1282	1301
445526	1283	1300	TGGTGCACAG TTTCTGGC	81	5/8/2005	249	1282	1299
416855	1284	1303	CATTGGTGCA CAGTTTCTGG	85	5/10/2005	220	1283	1302
445502	1284	1303	CATTGGTGCA CAGTTTCTGG	83	6/8/2006	220	1283	1302
445527	1284	1301	TTGGTGCACA GTTTCTGG	70	5/8/2005	250	1283	1300

412224	1285	1304	GCATTGGTGC ACAGTTTCTG	84	5/10/2005	52	1284	1303
445503	1285	1304	GCATTGGTGC ACAGTTTCTG	89	6/8/2006	52	1284	1303
445528	1285	1302	ATTGGTGCAC AGTTTCTG	73	5/8/2005	251	1284	1301
416856	1286	1305	GGCATTGGTG CACAGTTTCT	84	5/10/2005	221	1285	1304
445504	1286	1305	GGCATTGGTG CACAGTTTCT	87	6/8/2006	221	1285	1304
445529	1286	1303	CATTGGTGCA CAGTTTCT	85	5/8/2005	252	1285	1302
416857	1287	1306	CGGCATTGGT GCACAGTTTC	91	5/10/2005	222	1286	1305
445505	1287	1306	CGGCATTGGT GCACAGTTTC	89	6/8/2006	222	1286	1305
445530	1287	1304	GCATTGGTGC ACAGTTTC	83	5/8/2005	253	1286	1303
445506	1288	1307	ACGGCATTGG TGCACAGTTT	86	6/8/2006	223	1287	1306
445531	1288	1305	GGCATTGGTG CACAGTTT	90	5/8/2005	254	1287	1304
416859	1289	1308	GACGGCATTG GTGCACAGTT	85	5/10/2005	224	1288	1307
445507	1289	1308	GACGGCATTG GTGCACAGTT	85	6/8/2006	224	1288	1307
445532	1289	1306	CGGCATTGGT GCACAGTT	89	5/8/2005	255	1288	1305
413482	1290	1309	GGACGGCATT GGTGCACAGT	88	5/10/2005	115	1289	1308
445508	1290	1309	GGACGGCATT GGTGCACAGT	81	6/8/2006	115	1289	1308
445533	1290	1307	ACGGCATTGG TGCACAGT	87	5/8/2005	256	1289	1306
416860	1291	1310	CGGACGGCAT TGGTGCACAG	89	5/10/2005	225	1290	1309
445509	1291	1310	CGGACGGCAT TGGTGCACAG	84	6/8/2006	225	1290	1309
445534	1291	1308	GACGGCATTG GTGCACAG	82	5/8/2005	257	1290	1307

416861	1292	1311	GCGGACGGCA TTGGTGCACA	90	5/10/2005	226	1291	1310
445510	1292	1311	GCGGACGGCA TTGGTGCACA	88	6/8/2006	226	1291	1310
445535	1292	1309	GGACGGCATT GGTGCACA	83	5/8/2005	258	1291	1308
416862	1293	1312	AGCGGACGGC ATTGGTGCAC	89	5/10/2005	227	1292	1311
445511	1293	1312	AGCGGACGGC ATTGGTGCAC	77	6/8/2006	227	1292	1311
445536	1293	1310	CGGACGGCAT TGGTGCAC	82	5/8/2005	259	1292	1309
416863	1294	1313	CAGCGGACGG CATTGGTGCA	86	5/10/2005	228	1293	1312
445512	1294	1313	CAGCGGACGG CATTGGTGCA	79	6/8/2006	228	1293	1312
445537	1294	1311	GCGGACGGCA TTGGTGCA	78	5/8/2005	260	1293	1310
412225	1295	1314	GCAGCGGACG GCATTGGTGC	86	5/10/2005	53	1294	1313
445513	1295	1314	GCAGCGGACG GCATTGGTGC	85	6/8/2006	53	1294	1313
445538	1295	1312	AGCGGACGGC ATTGGTGC	80	5/8/2005	261	1294	1311
416864	1296	1315	GGCAGCGGAC GGCATTGGTG	88	5/10/2005	229	1295	1314
445514	1296	1315	GGCAGCGGAC GGCATTGGTG	81	6/8/2006	229	1295	1314
445539	1296	1313	CAGCGGACGG CATTGGTG	79	5/8/2005	262	1295	1312
416865	1297	1316	TGGCAGCGGA CGGCATTGGT	86	5/10/2005	230	1296	1315
445515	1297	1316	TGGCAGCGGA CGGCATTGGT	75	6/8/2006	230	1296	1315
445540	1297	1314	GCAGCGGACG GCATTGGT	74	5/8/2005	263	1296	1313
416866	1298	1317	CTGGCAGCGG ACGGCATTGG	84	5/10/2005	231	1297	1316
445516	1298	1317	CTGGCAGCGG ACGGCATTGG	79	6/8/2006	231	1297	1316

445541	1298	1315	GGCAGCGGAC GGCATTGG	80	5/8/2005	264	1297	1314
416867	1299	1318	ACTGGCAGCG GACGGCATTG	85	5/10/2005	232	1298	1317
445517	1299	1318	ACTGGCAGCG GACGGCATTG	74	6/8/2006	232	1298	1317
445542	1299	1316	TGGCAGCGGA CGGCATTG	83	5/8/2005	265	1298	1315
445543	1300	1317	CTGGCAGCGG ACGGCATT	74	5/8/2005	266	1299	1316

Example 31: Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells

Gapmers from Example 30 exhibiting *in vitro* inhibition of human Factor 11 were tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 123.46 nM, 370.37 nM, 1,111.11 nM, 3,333.33 nM and 10,000 nM concentrations of antisense oligonucleotide, as specified in Table 45. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells. As illustrated in Table 45, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide was calculated by plotting the concentrations of antisense oligonucleotides used versus the percent inhibition of Factor 11 mRNA expression achieved at each concentration, and noting the concentration of antisense oligonucleotide at which 50% inhibition of Factor 11 mRNA expression was achieved compared to the PBS control. IC_{50} values are presented in Table 45.

Table 45

Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells via transfection of oligonucleotides using electroporation

ISIS No.	123.47 nM	370.37 nM	1,111.11 nM	3,333.33 nM	10,000.0 nM	IC_{50} (μM)
416849	5	5	26	57	68	2.7
416850	0	12	36	74	73	2.8
416851	13	35	36	64	72	1.5

416856	12	23	35	59	83	1.6
416857	2	20	35	62	72	2.3
416858	0	27	36	64	70	2.2
416860	0	28	39	41	40	n.d.
416861	0	15	27	66	80	2.0
445498	3	1	27	50	58	4.8
445503	0	0	22	36	60	5.9
445504	8	20	38	53	68	2.7
445505	12	30	39	59	77	1.8
445522	0	0	44	63	74	2.9
445531	8	16	52	61	77	1.8
445532	5	12	39	60	70	2.0

n.d. =no data

Example 32: Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells by oligonucleotides designed by microwalk

Additional gapmers were designed based on ISIS 416850 and ISIS 416858 (see Table 8 above). These gapmers are shifted slightly upstream and downstream (i.e. microwalk) of ISIS 416850 and ISIS 416858. Gapmers designed by microwalk have 3-8-3 MOE, 4-8-4 MOE, 2-10-2 MOE, 3-10-3 MOE, or 4-10-4 MOE motifs.

These gapmers were tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 375 nM, 750 nM, 1,500 nM, 3,000 nM, 6,000 nM and 12,000 nM concentrations of antisense oligonucleotide, as specified in Table 47. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor 11 mRNA levels were measured by quantitative real-time PCR. Human Factor 11 primer probe set RTS 2966 was used to measure mRNA levels. Factor 11 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of Factor 11, relative to untreated control cells.

ISIS 416850, ISIS 416858, ISIS 445522, and ISIS 445531 (see Table 45 above) were re-tested *in vitro* along with the microwalk gapmers under the same conditions described above.

The chimeric antisense oligonucleotides in Table 46 were designed as 3-8-3 MOE, 4-8-4 MOE, 2-10-2 MOE, 3-10-3 MOE, or 4-10-4 MOE gapmers. The 3-8-3 gapmer is 14 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three nucleotides each. The 4-8-4 gapmer is 16 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising four nucleotides each. The 2-10-2 gapmer is 14 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising two nucleotides each. The 3-10-3 gapmer is 16 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three nucleotides each. The 4-10-4 gapmer is 18 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising four nucleotides each. For each of the motifs (3-8-3, 4-8-4, 2-10-2, 3-10-3, and 4-10-4), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Human Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the human sequence. "Human Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted in the human sequence. Each gapmer listed in Table 46 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3). Each gapmer is Table 46 is also fully cross-reactive with the rhesus monkey Factor 11 gene sequence, designated herein as SEQ ID NO: 274 (exons 1-15 GENBANK Accession No. NW_001118167.1). 'Rhesus monkey start site' indicates the 5'-most nucleotide to which the gapmer is targeted in the rhesus monkey sequence. 'Rhesus monkey stop site' indicates the 3'-most nucleotide to which the gapmer is targeted to the rhesus monkey sequence.

Table 46

Chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000128.3) and designed by microwalk of ISIS 416850 and ISIS 416858

ISIS No.	Human Target Start Site	Human Target Stop Site	Sequence (5' to 3')	Motif	SEQ ID No.	Rhesus monkey Start Site	Rhesus monkey Stop Site
449707	1280	1295	CACAGTTT CTGGCAGG	4-8-4	268	1279	1294
449708	1281	1294	ACAGTTT CTGGCAG	3-8-3	269	1280	1293
449709	1279	1296	GCACAGTT TCTGGCAGGC	4-10-4	246	1278	1295
449710	1280	1295	CACAGTTT CTGGCAGG	3-10-3	268	1279	1294

449711	1281	1294	ACAGTTT CTGGCAG	2-10-2	269	1280	1293

Dose-response inhibition data is given in Table 47. As illustrated in Table 47, Factor 11 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. The IC_{50} of each antisense oligonucleotide was also calculated and presented in Table 47. The first two listed gapmers in Table 47 are the original gapmers (ISIS 416850 and ISIS 416858) from which the remaining gapmers were designed via microwalk and are designated by an asterisk.

Table 47
Dose-dependent antisense inhibition of human Factor 11 in HepG2 cells via transfection of oligonucleotides using electroporation

ISIS No.	375 nM	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
*416850	40	59	69	87	90	95	0.56
*416858	31	35	78	85	90	93	0.83
445522	59	71	83	82	81	92	n.d.
445531	44	64	78	86	91	93	0.44
449707	7	35	63	73	85	91	1.26
449708	0	0	22	33	61	85	4.46
449709	52	71	80	87	92	95	0.38
449710	2	21	52	70	82	87	1.59
449711	6	14	1	7	32	52	11.04

n.d. =no data

Example 33: Tolerability of antisense oligonucleotides targeting human Factor 11 in CD1 mice

CD1 mice were treated with ISIS antisense oligonucleotides targeting human Factor 11 and evaluated for changes in the levels of various metabolic markers.

Treatment

Groups of five CD1 mice each were injected subcutaneously twice a week for 2, 4, or 6 weeks with 50 mg/kg of ISIS 416825, ISIS 416826, ISIS 416838, ISIS 416850, ISIS 416858, ISIS 416864, ISIS 416892, ISIS 416925, ISIS 416999, ISIS 417002, or ISIS 417003. A control group of five mice was injected subcutaneously with PBS for 2 weeks. All experimental groups (i.e. ASO treated mice at 2, 4, 6 weeks) were compared to the control group (i.e. PBS, 2 weeks).

Three days after the last dose was administered to all groups, the mice were sacrificed. Organ weights were measured and blood was collected for further analysis.

Organ weight

Liver, spleen, and kidney weights were measured at the end of the study, and are presented in Tables 48, 49, and 50 as a percent of the PBS control, normalized to body weight. Those antisense oligonucleotides which did not affect more than six-fold increases in liver and spleen weight above the PBS controls were selected for further studies.

Table 48

Percent change in liver weight of CD1 mice after antisense oligonucleotide treatment

ISIS No.	2 weeks	4 weeks	6 weeks
416825	+5	+22	+13
416826	+10	+32	+33
416838	+8	-6	0
416850	+5	+3	+6
416858	+7	+1	+10
416864	-2	+2	-5
416925	+14	+14	+33
416999	+13	+30	+47
417002	+14	+8	+35
416892	+35	+88	+95
417003	+8	+42	+32

Table 49

Percent change in spleen weight of CD1 mice after antisense oligonucleotide treatment

ISIS No.	2 weeks	4 weeks	6 weeks
416825	-12	+19	+21
416826	-12	-5	+22
416838	+21	-8	+9
416850	-4	+6	+48
416858	-2	+8	+28
416864	-10	-2	-6
416925	-7	+33	+78
416999	+7	+22	+38
417002	+29	+26	+108
416892	+24	+30	+65
417003	+12	+101	+98

Table 50
Percent change in kidney weight of CD1 mice after antisense oligonucleotide treatment

ISIS No.	2 weeks	4 weeks	6 weeks
416825	-12	-12	-11
416826	-13	-7	-22
416838	-2	-12	-8
416850	-10	-12	-11
416858	+1	-18	-10
416864	-4	-9	-15
416925	-4	-14	-2
416999	-9	-6	-7
417002	+3	-5	-2
416892	+2	-3	+19
417003	-9	-2	-1

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Measurements of alanine transaminase (ALT) and aspartate transaminase (AST) are expressed in IU/L and the results are presented in Tables 51 and 52. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer and expressed in mg/dL. The results are presented in Tables 53 and 54. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Those antisense oligonucleotides which did not increase levels of bilirubin more than two-fold of the control levels were selected for further studies.

Table 51
Effect of antisense oligonucleotide treatment on ALT (IU/L) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	36	n.d.	n.d.
ISIS 416825	64	314	507
ISIS 416826	182	126	1954
ISIS 416838	61	41	141
ISIS 416850	67	58	102
ISIS 416858	190	57	216
ISIS 416864	44	33	92
ISIS 416925	160	284	1284
ISIS 416999	61	160	1302
ISIS 417002	71	138	2579
ISIS 416892	66	1526	1939

ISIS 417003	192	362	2214

n.d.= no data

Table 52

Effect of antisense oligonucleotide treatment on AST (IU/L) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	68	n.d.	n.d.
ISIS 416825	82	239	301
ISIS 416826	274	156	1411
ISIS 416838	106	73	107
ISIS 416850	72	88	97
ISIS 416858	236	108	178
ISIS 416864	58	46	101
ISIS 416925	144	206	712
ISIS 416999	113	130	671
ISIS 417002	96	87	1166
ISIS 416892	121	1347	1443
ISIS 417003	152	249	839

n.d.= no data

Table 53

Effect of antisense oligonucleotide treatment on bilirubin (mg/dL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	0.28	n.d.	n.d.
ISIS 416825	0.41	0.69	0.29
ISIS 416826	0.39	0.20	0.37
ISIS 416838	0.57	0.24	0.20
ISIS 416850	0.46	0.23	0.22
ISIS 416858	0.57	0.24	0.16
ISIS 416864	0.40	0.26	0.22
ISIS 416925	0.45	0.25	0.25
ISIS 416999	0.48	0.18	0.28
ISIS 417002	0.50	0.25	0.29
ISIS 416892	0.38	2.99	0.50
ISIS 417003	0.33	0.15	0.24

n.d.= no data

Table 54

Effect of antisense oligonucleotide treatment on albumin (mg/dL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	3.7	n.d.	n.d.
ISIS 416825	3.6	3.4	3.5
ISIS 416826	3.3	3.4	3.4

ISIS 416838	3.5	3.8	3.6
ISIS 416850	3.6	3.5	3.1
ISIS 416858	3.4	3.5	2.8
ISIS 416864	3.5	3.6	3.5
ISIS 416925	3.5	3.5	3.2
ISIS 416999	3.4	3.3	3.2
ISIS 417002	3.2	3.4	3.4
ISIS 416892	3.2	4.0	4.4
ISIS 417003	3.4	3.4	3.2

n.d.= no data

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Tables 55 and 56, expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies.

Table 55

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	30	n.d.	n.d.
ISIS 416825	29	35	31
ISIS 416826	24	34	27
ISIS 416838	25	38	30
ISIS 416850	25	30	23
ISIS 416858	21	29	19
ISIS 416864	22	31	28
ISIS 416925	21	30	17
ISIS 416999	22	27	22
ISIS 417002	19	23	19
ISIS 416892	19	28	23
ISIS 417003	23	26	24

n.d.= no data

Table 56

Effect of antisense oligonucleotide treatment on creatinine (mg/dL) in CD1 mice

	2 weeks	4 weeks	6 weeks

PBS	0.14	n.d.	n.d.
ISIS 416825	0.14	0.21	0.17
ISIS 416826	0.15	0.20	0.15
ISIS 416838	0.09	0.27	0.14
ISIS 416850	0.13	0.22	0.19
ISIS 416858	0.13	0.23	0.10
ISIS 416864	0.11	0.22	0.16
ISIS 416925	0.12	0.25	0.13
ISIS 416999	0.07	0.18	0.13
ISIS 417002	0.06	0.16	0.10
ISIS 416892	0.11	0.20	0.17
ISIS 417003	0.17	0.24	0.18

n.d.= no data

Hematology assays

Blood obtained from all mice groups were sent to Antech Diagnostics for hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) measurements and analyses, as well as measurements of the various blood cells, such as WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 57-67. Percentages given in the tables indicate the percent of total blood cell count. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and/or an increase in monocyte count of more than three-fold were selected for further studies.

Table 57

Effect of antisense oligonucleotide treatment on HCT (%) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	50	n.d.	n.d.
ISIS 416825	49	46	40
ISIS 416826	47	41	37
ISIS 416838	42	44	39
ISIS 416850	44	44	38
ISIS 416858	50	45	46
ISIS 416864	50	45	42
ISIS 416925	51	47	47
ISIS 416999	51	42	40
ISIS 417002	44	44	51
ISIS 416892	48	42	45
ISIS 417003	48	41	43

n.d.= no data

Table 58
Effect of antisense oligonucleotide treatment on MCV (fL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	61	n.d.	n.d.
ISIS 416825	58	53	51
ISIS 416826	56	52	53
ISIS 416838	56	54	48
ISIS 416850	57	51	50
ISIS 416858	59	51	50
ISIS 416864	57	52	51
ISIS 416925	61	52	47
ISIS 416999	60	49	48
ISIS 417002	61	50	52
ISIS 416892	59	49	53
ISIS 417003	60	48	45

n.d.= no data

Table 59
Effect of antisense oligonucleotide treatment on MCH (pg) in CD1 mice

ISIS No.	2 weeks	4 weeks	6 weeks
PBS	18	n.d.	n.d.
ISIS 416825	17	16	15
ISIS 416826	17	16	16
ISIS 416838	17	17	15
ISIS 416850	17	16	15
ISIS 416858	17	16	15
ISIS 416864	18	16	16
ISIS 416925	17	16	15
ISIS 416999	17	16	15
ISIS 417002	17	16	16
ISIS 416892	18	16	16
ISIS 417003	17	16	16

n.d.= no data

Table 60
Effect of antisense oligonucleotide treatment on MCHC (%) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	30	n.d.	n.d.
ISIS 416825	29	31	31
ISIS 416826	29	31	30

ISIS 416838	30	31	32
ISIS 416850	30	31	31
ISIS 416858	30	32	31
ISIS 416864	31	31	31
ISIS 416925	30	32	32
ISIS 416999	27	32	31
ISIS 417002	29	32	31
ISIS 416892	30	32	30
ISIS 417003	29	32	33

n.d.= no data

Table 61

Effect of antisense oligonucleotide treatment on WBC count (cells/nL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	6	n.d.	n.d.
ISIS 416825	8	8	6
ISIS 416826	5	6	8
ISIS 416838	4	6	5
ISIS 416850	4	5	5
ISIS 416858	6	7	4
ISIS 416864	7	6	5
ISIS 416925	6	6	11
ISIS 416999	4	9	7
ISIS 417002	8	8	16
ISIS 416892	5	8	9
ISIS 417003	7	9	10

n.d.= no data

Table 62

Effect of antisense oligonucleotide treatment on RBC count (cells/pL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	8	n.d.	n.d.
ISIS 416825	9	9	8
ISIS 416826	8	8	7
ISIS 416838	8	8	8
ISIS 416850	8	9	8
ISIS 416858	9	9	9
ISIS 416864	9	9	8
ISIS 416925	9	9	10
ISIS 416999	9	9	8
ISIS 417002	9	9	10
ISIS 416892	7	9	9

ISIS 417003	8	9	10

n.d.= no data

Table 63

Effect of antisense oligonucleotide treatment on neutrophil count (%) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	16	n.d.	n.d.
ISIS 416825	15	43	23
ISIS 416826	26	33	23
ISIS 416838	19	33	31
ISIS 416850	15	21	16
ISIS 416858	14	24	27
ISIS 416864	13	27	20
ISIS 416925	12	39	33
ISIS 416999	12	25	22
ISIS 417002	14	31	36
ISIS 416892	19	43	28
ISIS 417003	10	39	24

n.d.= no data

Table 64

Effect of antisense oligonucleotide treatment on lymphocyte count (%) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	81	n.d.	n.d.
ISIS 416825	82	53	71
ISIS 416826	70	61	67
ISIS 416838	76	64	60
ISIS 416850	82	73	76
ISIS 416858	83	73	65
ISIS 416864	84	71	74
ISIS 416925	86	58	57
ISIS 416999	86	72	69
ISIS 417002	83	64	51
ISIS 416892	79	52	64
ISIS 417003	86	54	66

n.d.= no data

Table 65

Effect of antisense oligonucleotide treatment on monocyte count (%) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	3	n.d.	n.d.
ISIS 416825	2	5	4

ISIS 416826	3	5	8
ISIS 416838	2	2	6
ISIS 416850	3	6	6
ISIS 416858	2	3	7
ISIS 416864	2	2	5
ISIS 416925	2	4	8
ISIS 416999	2	4	8
ISIS 417002	3	4	12
ISIS 416892	3	6	7
ISIS 417003	2	6	8

n.d.= no data

Table 66

Effect of antisense oligonucleotide treatment on platelet count (cells/nL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	2126	n.d.	n.d.
ISIS 416825	1689	1229	942
ISIS 416826	1498	970	645
ISIS 416838	1376	1547	1229
ISIS 416850	1264	1302	1211
ISIS 416858	2480	1364	1371
ISIS 416864	1924	1556	933
ISIS 416925	1509	1359	1211
ISIS 416999	1621	1219	1057
ISIS 417002	1864	1245	1211
ISIS 416892	1687	636	1004
ISIS 417003	1309	773	922

n.d.= no data

Table 67

Effect of antisense oligonucleotide treatment on hemoglobin content (g/dL) in CD1 mice

	2 weeks	4 weeks	6 weeks
PBS	15.1	n.d.	n.d.
ISIS 416825	14.5	14.1	12.1
ISIS 416826	13.4	12.8	11.0
ISIS 416838	12.4	13.6	12.6
ISIS 416850	13.1	13.5	11.6
ISIS 416858	14.8	14.2	14.1
ISIS 416864	15.2	13.9	13.0
ISIS 416925	14.9	14.8	15.3
ISIS 416999	14.2	13.3	12.8
ISIS 417002	14.7	14.0	15.7

ISIS 416892	13.0	13.5	13.1
ISIS 417003	13.7	13.4	14.0

n.d.= no data

Example 34: Measurement of half-life of antisense oligonucleotide in CD1 mice liver

CD1 mice were treated with ISIS antisense oligonucleotides targeting human Factor 11 and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver was evaluated.

Treatment

Groups of fifteen CD1 mice each were injected subcutaneously twice per week for 2 weeks with 50 mg/kg of ISIS 416825, ISIS 416826, ISIS 416838, ISIS 416850, ISIS 416858, ISIS 416864, ISIS 416892, ISIS 416925, ISIS 416999, ISIS 417002, or ISIS 417003. Five mice from each group were sacrificed 3 days, 28 days and 56 days following the final dose. Livers were harvested for analysis.

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 270) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 µg/g. Half-lives were then calculated using WinNonlin software (PHARSIGHT).

The results are presented in Tables 68 and 69, expressed as µg/g liver tissue. The half-life of each oligonucleotide is presented in Table 70.

Table 68

Full-length oligonucleotide concentration (µg/g) in the liver of CD1 mice

ISIS No.	Motif	day 3	day 28	day 56
416825	5-10-5	151	52	7
416826	5-10-5	186	48	8

416838	5-10-5	170	46	10
416850	5-10-5	238	93	51
416858	5-10-5	199	102	18
416864	5-10-5	146	38	25
416999	2-13-5	175	26	0
417002	2-13-5	119	24	1
417003	2-13-5	245	42	4
416925	3-14-3	167	39	5
416892	3-14-3	135	31	6

Table 69

Total oligonucleotide concentration (μg/g) in the liver of CD1 mice

ISIS No.	Motif	day 3	day 28	day 56
416825	5-10-5	187	90	39
416826	5-10-5	212	61	12
416838	5-10-5	216	98	56
416850	5-10-5	295	157	143
416858	5-10-5	273	185	56
416864	5-10-5	216	86	112
416999	2-13-5	232	51	0
417002	2-13-5	206	36	1
417003	2-13-5	353	74	4
416925	3-14-3	280	72	8
416892	3-14-3	195	54	6

Table 70

Half-life of antisense oligonucleotides in the liver of CD1 mice

ISIS No.	Motif	Half-life (days)
416825	5-10-5	16
416826	5-10-5	13
416838	5-10-5	13
416850	5-10-5	18
416858	5-10-5	26
416864	5-10-5	13
416999	2-13-5	9
417002	2-13-5	11
417003	2-13-5	10
416925	3-14-3	12
416892	3-14-3	12

Example 35: Tolerability of antisense oligonucleotides targeting human Factor 11 in Sprague-Dawley rats

Sprague-Dawley rats were treated with ISIS antisense oligonucleotides targeting human Factor 11 and evaluated for changes in the levels of various metabolic markers.

Treatment

Groups of four Sprague Dawley rats each were injected subcutaneously twice per week for 6 weeks with 50 mg/kg of ISIS 416825, ISIS 416826, ISIS 416838, ISIS 416850, ISIS 416858, ISIS 416848, ISIS 416864, ISIS 416892, ISIS 416925, ISIS 416999, ISIS 417002, or ISIS 417003. A control group of four Sprague Dawley rats was injected subcutaneously with PBS twice per week for 6 weeks. Body weight measurements were taken before and throughout the treatment period. Urine samples were taken before the start of treatment. Three days after the last dose, urine samples were taken and the rats were sacrificed. Organ weights were measured and blood was collected for further analysis.

Body weight and organ weight

Body weights of the rats were measured at the onset of the study and subsequently twice per week. The body weights are presented in Table 71 and are expressed as a percent change over the weights taken at the start of the study. Liver, spleen, and kidney weights were measured at the end of the study and are presented in Table 71 as a percent of the saline control normalized to body weight. Those antisense oligonucleotides which did not affect more than a six-fold increase in liver and spleen weight above the PBS control were selected for further studies.

Table 71

Percent change in organ weight of Sprague Dawley rats after antisense oligonucleotide treatment

ISIS No.	Liver	Spleen	Kidney	Body weight
416825	+20	+245	+25	-18
416826	+81	+537	+44	-40
416838	+8	+212	-0.5	-23
416850	+23	+354	+47	-33
416858	+8	+187	+5	-21
416864	+16	+204	+16	-24
416925	+44	+371	+48	-32

416999	+51	+405	+71	-37
417002	+27	+446	+63	-29
416892	+38	+151	+32	-39
417003	+51	+522	+25	-40

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Measurements of alanine transaminase (ALT) and aspartate transaminase (AST) are expressed in IU/L and the results are presented in Table 72. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin and albumin were also measured with the same clinical analyzer and the results are also presented in Table 72, expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies.

Table 72

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of Sprague-Dawley rats

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)
PBS	9	5	20	2
ISIS 416825	89	17	4	2
ISIS 416826	611	104	115	6
ISIS 416838	5	2	4	2
ISIS 416850	80	5	1	4
ISIS 416858	13	4	4	2
ISIS 416864	471	68	3	4
ISIS 416925	102	20	13	5
ISIS 416999	92	28	54	5
ISIS 417002	44	11	12	3
ISIS 416892	113	183	1	8
ISIS 417003	138	23	50	6

Kidney function

To evaluate the effect of kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 73, expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies. The ratio of urine protein to creatinine in total urine samples was also calculated before and after antisense oligonucleotide treatment and is presented in Table 74. Those antisense oligonucleotides which did not affect more than a five-fold increase in urine protein/creatinine ratios compared to the PBS control were selected for further studies.

Table 73

Effect of antisense oligonucleotide treatment on metabolic markers in the kidney of Sprague-Dawley rats

	BUN	Creatinine
PBS	4	8
ISIS 416825	7	17
ISIS 416826	25	6
ISIS 416838	4	5
ISIS 416850	5	7
ISIS 416858	8	4
ISIS 416864	5	6
ISIS 416925	7	5
ISIS 416999	2	4
ISIS 417002	11	1
ISIS 416892	188	1
ISIS 417003	9	9

Table 74

Effect of antisense oligonucleotide treatment on urine protein/creatinine ratio in Sprague Dawley rats

	Before	After
PBS	1.2	1.3
416825	1.1	5.4
416826	1.0	11.4
416838	1.2	3.7
416850	1.0	4.0
416858	0.9	4.4
416864	1.2	4.0
416925	1.0	4.3
416999	1.3	9.1
417002	1.0	2.4
416892	0.8	21.3
417003	0.9	4.8

Hematology assays

Blood obtained from all rat groups were sent to Antech Diagnostics for hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCV), and mean corpuscular hemoglobin concentration (MCHC)measurements and analyses, as well as measurements of various blood cells, such as WBC (neutrophils, lymphocytes and monocytes), RBC, and platelets as well as hemoglobin content. The results are presented in Tables 75 and 76. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies.

Table 75

Effect of antisense oligonucleotide treatment on blood cell count in Sprague-Dawley rats

	WBC (/nL)	RBC (/pL)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Platelets ($10^3/\mu L$)
PBS	21	6	37	7	26	18
ISIS 416825	22	2	25	3	15	6
ISIS 416826	7	5	30	5	7	11
ISIS 416838	13	4	17	3	6	27
ISIS 416850	16	7	48	8	11	26
ISIS 416858	28	2	20	3	10	19
ISIS 416864	15	4	26	2	29	12
ISIS 416925	24	6	20	4	23	8
ISIS 416999	12	5	23	3	20	12
ISIS 417002	23	5	22	4	25	7
ISIS 416892	68	12	92	18	58	66
ISIS 417003	83	11	17	3	6	19

Table 76

Effect of antisense oligonucleotide treatment on hematologic factors (% control) in Sprague-Dawley rats

	Hemoglobin (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (%)
PBS	6	4	6	2	4
ISIS 416825	2	2	4	2	4
ISIS 416826	7	7	6	3	4
ISIS 416838	2	5	4	2	5
ISIS 416850	4	5	3	4	2
ISIS 416858	2	3	2	2	1
ISIS 416864	4	2	4	2	4
ISIS 416925	6	8	5	2	4
ISIS 416999	6	5	2	3	1
ISIS 417002	5	7	7	3	5

ISIS 416892	14	13	1	2	0
ISIS 417003	11	8	6	4	4

Example 36: Measurement of half-life of antisense oligonucleotide in Sprague-Dawley rat liver and kidney

Sprague Dawley rats were treated with ISIS antisense oligonucleotides targeting human Factor 11 and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver and kidney was evaluated.

Treatment

Groups of four Sprague Dawley rats each were injected subcutaneously twice a week for 2 weeks with 20 mg/kg of ISIS416825, ISIS 416826, ISIS 416838, ISIS 416850, ISIS 416858, ISIS 416864, ISIS 416892, ISIS 416925, ISIS 416999, ISIS 417002, or ISIS 417003. Three days after the last dose, the rats were sacrificed and livers and kidneys were collected for analysis.

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 270) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. The results are presented in Tables 77 and 78, expressed as μg/g liver or kidney tissue. Half-lives were then calculated using WinNonlin software (PHARSIGHT).

Table 77

Full-length oligonucleotide concentration (μg/g) in the liver and kidney of Sprague-Dawley rats

ISIS No.	Motif	Kidney	Liver
416825	5-10-5	632	236
416826	5-10-5	641	178
416838	5-10-5	439	171
416850	5-10-5	259	292
416858	5-10-5	575	255

416864	5-10-5	317	130
416999	2-13-5	358	267
417002	2-13-5	291	118
417003	2-13-5	355	199
416925	3-14-3	318	165
416892	3-14-3	351	215

Table 78

Total oligonucleotide concentration (µg/g) in the liver and kidney of Sprague-Dawley rats

ISIS No.	Motif	Kidney	Liver
416825	5-10-5	845	278
416826	5-10-5	775	214
416838	5-10-5	623	207
416850	5-10-5	352	346
416858	5-10-5	818	308
416864	5-10-5	516	209
416999	2-13-5	524	329
417002	2-13-5	490	183
417003	2-13-5	504	248
416925	3-14-3	642	267
416892	3-14-3	608	316

Table 79

Half-life (days) of ISIS oligonucleotides in the liver and kidney of Sprague-Dawley rats

ISIS No.	Motif	Half-life
416825	5-10-5	16
416826	5-10-5	13
416838	5-10-5	13
416850	5-10-5	18
416858	5-10-5	26
416864	5-10-5	13
416999	2-13-5	9
417002	2-13-5	11
417003	2-13-5	10
416925	3-14-3	12
416892	3-14-3	12

Example 37: Tolerability of antisense oligonucleotides targeting human Factor 11 in CD1 mice

CD1 mice were treated with ISIS antisense oligonucleotides targeting human Factor 11 and evaluated for changes in the levels of various metabolic markers.

Treatment

Groups of five CD1 mice each were injected subcutaneously twice per week for 6 weeks with 50 mg/kg of ISIS 412223, ISIS 412224, ISIS 412225, ISIS 413481, ISIS 413482, ISIS 416848, ISIS 416849, ISIS 416850, ISIS 416851, ISIS 416852, ISIS 416853, ISIS 416854, ISIS 416855, ISIS 416856, ISIS 416857, ISIS 416858, ISIS 416859, ISIS 416860, ISIS 416861, ISIS 416862, ISIS 416863, ISIS 416864, ISIS 416865, ISIS 416866, or ISIS 416867, or. A control group of ten CD1 mice was injected subcutaneously with PBS twice per week for 6 weeks. Body weight measurements were taken before and throughout the treatment period. Three days after the last dose, the mice were sacrificed, organ weights were measured, and blood was collected for further analysis.

Body weight and organ weights

Body weight was measured at the onset of the study and subsequently twice per week. The body weights of the mice are presented in Table 80 and are expressed increase in grams over the PBS control weight taken before the start of treatment. Liver, spleen, and kidney weights were measured at the end of the study, and are also presented in Table 80 as percentage of the body weight. Those antisense oligonucleotides which did not affect more than six-fold increases in liver and spleen weight above the PBS control were selected for further studies.

Table 80

Change in body and organ weights of CD1 mice after antisense oligonucleotide treatment

	Liver (%)	Kidney (%)	Spleen (%)	body weight (g)
PBS	5	1.5	0.3	7
ISIS 416850	6	1.6	0.4	12
ISIS 416858	7	1.6	0.6	12
ISIS 416864	5	1.6	0.3	12
ISIS 412223	6	1.5	0.4	12
ISIS 412224	6	1.6	0.5	10
ISIS 412225	6	1.5	0.4	10
ISIS 413481	6	1.5	0.5	9

ISIS 413482	6	1.6	0.5	11
ISIS 416848	6	1.5	0.4	11
ISIS 416849	8	1.5	0.4	8
ISIS 416851	7	1.5	0.5	11
ISIS 416852	6	1.5	0.4	10
ISIS 416853	8	1.5	0.7	13
ISIS 416854	7	1.2	0.4	13
ISIS 416855	8	1.4	0.6	12
ISIS 416856	6	1.4	0.4	10
ISIS 416857	7	1.6	0.5	10
ISIS 416859	6	1.5	0.4	10
ISIS 416860	6	1.4	0.4	10
ISIS 416861	5	1.3	0.4	9
ISIS 416862	6	1.5	0.4	10
ISIS 416863	5	1.5	0.4	9
ISIS 416865	6	1.5	0.4	8
ISIS 416866	5	1.6	0.4	10
ISIS 416867	5	1.4	0.4	9

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Measurements of alanine transaminase (ALT) and aspartate transaminase (AST) are expressed in IU/L and the results are presented in Table 81. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin, cholesterol and albumin were also measured using the same clinical chemistry analyzer and are presented in Table 81 expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies.

Table 81

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of CD1 mice

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)	Cholesterol (mg/dL)
PBS	32	68	0.25	3.7	135
ISIS 416850	75	99	0.21	3.5	142
ISIS 416858	640	547	0.28	4.4	181
ISIS 416864	36	67	0.19	2.6	152
ISIS 412223	60	125	0.20	3.0	117

ISIS 412224	214	183	0.19	3.4	114
ISIS 412225	40	69	0.23	3.3	128
ISIS 413481	85	143	0.18	3.2	153
ISIS 413482	54	77	0.24	3.0	138
ISIS 416848	153	153	0.19	3.1	151
ISIS 416849	1056	582	0.22	2.5	109
ISIS 416851	47	76	0.19	3.1	106
ISIS 416852	49	91	0.16	4.9	125
ISIS 416853	1023	1087	0.25	3.1	164
ISIS 416854	1613	1140	0.21	5.5	199
ISIS 416855	786	580	0.25	4.2	162
ISIS 416856	130	129	0.23	5.2	109
ISIS 416857	370	269	0.22	3.7	94
ISIS 416859	214	293	0.20	4.2	160
ISIS 416860	189	160	0.23	3.5	152
ISIS 416861	38	85	0.27	4.3	133
ISIS 416862	225	172	0.36	3.9	103
ISIS 416863	41	101	0.24	3.6	118
ISIS 416865	383	262	0.27	4.1	95
ISIS 416866	36	120	0.29	4.3	113
ISIS 416867	45	82	0.21	3.3	144

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer and results are presented in Table 82 expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies.

Table 82

Effect of antisense oligonucleotide treatment on BUN levels (mg/dL) in the kidney of CD1 mice

	BUN
PBS	22
ISIS 416850	24
ISIS 416858	23
ISIS 416864	24
ISIS 412223	28
ISIS 412224	29

ISIS 412225	23
ISIS 413481	23
ISIS 413482	27
ISIS 416848	23
ISIS 416849	23
ISIS 416851	21
ISIS 416852	21
ISIS 416853	22
ISIS 416854	27
ISIS 416855	23
ISIS 416856	21
ISIS 416857	17
ISIS 416859	18
ISIS 416860	25
ISIS 416861	23
ISIS 416862	21
ISIS 416863	22
ISIS 416865	20
ISIS 416866	22
ISIS 416867	20

Hematology assays

Blood obtained from all the mice groups were sent to Antech Diagnostics for hematocrit (HCT) measurements, as well as measurements of various blood cells, such as WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, as well as total hemoglobin content analysis. The results are presented in Tables 83 and 84. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies.

Table 83

Effect of antisense oligonucleotide treatment on hematologic factors in CD1 mice

	RBC ($10^6/\mu L$)	Hemoglobin (g/dL)	HCT (%)	WBC ($10^3/\mu L$)
PBS	10	15	51	7
ISIS 416850	10	15	49	5
ISIS 416858	9	14	50	8
ISIS 416864	10	15	52	5
ISIS 412223	9	15	48	7
ISIS 412224	10	15	50	9
ISIS 412225	9	15	50	7

ISIS 413481	9	13	45	7
ISIS 413482	10	15	50	8
ISIS 416848	9	14	47	7
ISIS 416849	9	14	48	9
ISIS 416851	9	14	47	6
ISIS 416852	9	14	49	5
ISIS 416853	11	17	56	8
ISIS 416854	9	13	43	12
ISIS 416855	9	14	50	6
ISIS 416856	9	14	47	5
ISIS 416857	10	15	53	6
ISIS 416859	10	15	49	6
ISIS 416860	10	15	51	7
ISIS 416861	9	14	48	7
ISIS 416862	9	14	49	6
ISIS 416863	9	14	48	7
ISIS 416865	9	14	50	7
ISIS 416866	9	15	51	6
ISIS 416867	10	14	47	8

Table 84

Effect of antisense oligonucleotide treatment on blood cell count in CD1 mice

	Neutrophil (cells/μL)	Lymphocyte (cells/μL)	Monocytes (cells/μL)	Platelets (10^3/μL)
PBS	1023	6082	205	940
ISIS 416850	1144	4004	156	916
ISIS 416858	2229	5480	248	782
ISIS 416864	973	3921	141	750
ISIS 412223	1756	4599	200	862
ISIS 412224	2107	6284	195	647
ISIS 412225	1547	4969	293	574
ISIS 413481	1904	4329	204	841
ISIS 413482	1958	5584	275	818
ISIS 416848	1264	5268	180	953
ISIS 416849	1522	6967	253	744
ISIS 416851	1619	4162	194	984
ISIS 416852	1241	3646	189	903

ISIS 416853	2040	5184	225	801
ISIS 416854	2082	9375	455	1060
ISIS 416855	1443	4236	263	784
ISIS 416856	1292	3622	151	753
ISIS 416857	1334	3697	215	603
ISIS 416859	1561	4363	229	826
ISIS 416860	1291	4889	161	937
ISIS 416861	1122	5119	219	836
ISIS 416862	1118	4445	174	1007
ISIS 416863	1330	5617	226	1131
ISIS 416865	1227	5148	315	872
ISIS 416866	1201	4621	211	1045
ISIS 416867	1404	6078	188	1006

Example 38: Measurement of half-life of antisense oligonucleotide in CD1 mouse liver

Fifteen antisense oligonucleotides which had been evaluated in CD1 mice (Example 37) were further evaluated. CD1 mice were treated with ISIS antisense oligonucleotides and the oligonucleotide half-life as well the elapsed time for oligonucleotide degradation and elimination in the liver was evaluated.

Treatment

Groups of fifteen CD1 mice each were injected subcutaneously twice per week for 2 weeks with 50 mg/kg of ISIS 412223, ISIS 412225, ISIS 413481, ISIS 413482, ISIS 416851, ISIS 416852, ISIS 416856, ISIS 416860, ISIS 416861, ISIS 416863, ISIS 416866, ISIS 416867, ISIS 412224, ISIS 416848 or ISIS 416859. Five mice from each group were sacrificed 3 days, 28 days, and 56 days after the last dose, livers were collected for analysis.

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 270) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. The results are presented in Table 85 expressed as μg/g liver tissue. The half-life of each oligonucleotide was also presented in Table 85.

Table 85

Full-length oligonucleotide concentration and half-life in the liver of CD1 mice

ISIS No	Motif	day 3	day 28	day 56	Half-Life (days)
412223	5-10-5	276	127	52	21.9
412224	5-10-5	287	111	31	16.6
412225	5-10-5	279	91	47	20.7
413481	5-10-5	185	94	31	20.6
413482	5-10-5	262	95	40	19.5
416848	5-10-5	326	147	68	23.5
416851	5-10-5	319	147	68	23.8
416852	5-10-5	306	145	83	28.4
416856	5-10-5	313	115	46	19.2
416859	5-10-5	380	156	55	19.0
416860	5-10-5	216	96	36	20.6
416861	5-10-5	175	59	39	24.5
416863	5-10-5	311	101	48	19.8
416866	5-10-5	246	87	25	16.0
416867	5-10-5	246	87	35	18.9

Example 39: Tolerability of antisense oligonucleotides targeting human Factor 11 in Sprague-Dawley rats

Fifteen antisense oligonucleotides which had been evaluated in CD1 mice (Example 37) were further evaluated in Sprague-Dawley rats for changes in the levels of various metabolic markers.

Treatment

Groups of four Sprague Dawley rats each were injected subcutaneously twice per week for 6 weeks with 50 mg/kg of ISIS 412223, ISIS 412224, ISIS 412225, ISIS 413481, ISIS 413482, ISIS 416848, ISIS 416851, ISIS 416852, ISIS 416856, ISIS 416859, ISIS 416860, ISIS 416861, ISIS 416863, ISIS 416866, or ISIS 416867 . A control group of four Sprague Dawley rats was injected subcutaneously with PBS twice per week for 6 weeks. Body weight measurements were taken before and throughout the treatment period. Three days after the last dose, urine samples were collected and the rats were then sacrificed, organ weights were measured, and blood was collected for further analysis.

Body weight and organ weights

The body weights of the rats were measured at the onset of the study and subsequently twice per week. The body weights are presented in Table 86 and are expressed as increase in grams over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 86 as a percentage of the body weight. Those antisense oligonucleotides which did not affect more than six-fold increases in liver and spleen weight above the PBS control were selected for further studies.

Table 86

Change in body and organ weights of Sprague Dawley rats after antisense oligonucleotide treatment

	Body weight (g)	Liver (%)	Kidney (%)	Spleen (%)
PBS	179	4	0.9	0.2
ISIS 412223	126	5	1.0	0.5
ISIS 412224	165	5	1.0	0.5
ISIS 412225	184	4	1.0	0.5
ISIS 413481	147	5	0.9	0.3
ISIS 413482	158	5	1.0	0.6
ISIS 416848	117	5	1.1	0.8
ISIS 416851	169	5	0.9	0.3
ISIS 416852	152	5	1.0	0.4
ISIS 416856	156	5	1.0	0.4
ISIS 416859	128	4	1.0	0.4
ISIS 416860	123	5	1.0	0.5
ISIS 416861	182	5	0.9	0.3
ISIS 416863	197	5	1.0	0.4
ISIS 416866	171	5	1.0	0.5
ISIS 416867	129	5	1.0	0.5

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Measurements of alanine transaminase (ALT) and aspartate transaminase

(AST) are expressed in IU/L and the results are presented in Table 87. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer and results are presented in Table 87 and expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies.

Table 87

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of Sprague-Dawley rats

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)
PBS	42	71	0.13	4
ISIS 412223	85	180	0.14	5
ISIS 412224	84	132	0.12	4
ISIS 412225	48	108	0.15	5
ISIS 413481	54	80	0.22	4
ISIS 413482	59	157	0.14	4
ISIS 416848	89	236	0.14	3
ISIS 416851	64	91	0.14	4
ISIS 416852	49	87	0.15	4
ISIS 416856	123	222	0.13	4
ISIS 416859	114	206	0.21	5
ISIS 416860	70	157	0.15	4
ISIS 416861	89	154	0.15	5
ISIS 416863	47	78	0.13	4
ISIS 416866	41	78	0.16	4
ISIS 416867	47	126	0.17	4

Kidney function

To evaluate the effect of ISIS oligonucleotides on the kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 88, expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies. The total urine protein and ratio of urine protein to creatinine in total urine samples after antisense oligonucleotide treatment was calculated and is also presented in Table 88. Those antisense oligonucleotides which did not affect more than a five-fold increase in urine protein/creatinine ratios compared to the PBS control were selected for further studies.

Table 88

Effect of antisense oligonucleotide treatment on metabolic markers in the kidney of Sprague-Dawley rats

	BUN (mg/dL)	Creatinine (mg/dL)	Total urine protein (mg/dL)	Urine protein/creatinine ratio
PBS	19	38	60	1.7
ISIS 412223	24	46	224	4.6
ISIS 412224	24	44	171	3.8
ISIS 412225	23	58	209	4.0
ISIS 413481	26	45	148	3.6
ISIS 413482	23	34	157	4.8
ISIS 416848	26	64	231	3.9
ISIS 416851	24	70	286	4.0
ISIS 416852	25	60	189	3.0
ISIS 416856	23	48	128	2.7
ISIS 416859	24	44	144	3.3
ISIS 416860	23	58	242	4.6
ISIS 416861	22	39	205	5.1
ISIS 416863	29	73	269	3.8
ISIS 416866	22	85	486	6.2
ISIS 416867	22	70	217	3.1

Hematology assays

Blood obtained from all rat groups were sent to Antech Diagnostics for hematocrit (HCT) measurements, as well as measurements of the various blood cells, such as WBC (neutrophils and lymphocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 89 and 90. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies.

Table 89

Effect of antisense oligonucleotide treatment on hematologic factors in Sprague-Dawley rats

	RBC (10^6/mL)	Hemoglobin (g/dL)	HCT (%)	WBC (10^3/mL)
PBS	6.9	13.2	42	9
ISIS 412223	7.2	13.1	41	20
ISIS 412224	7.4	13.4	42	20
ISIS 412225	7.4	13.4	42	15
ISIS 413481	7.5	14.2	43	14
ISIS 413482	7.1	13.2	40	13
ISIS 416848	6.0	11.1	35	17
ISIS 416851	7.4	13.7	42	11
ISIS 416852	7.2	13.4	42	13
ISIS 416856	7.7	14.1	43	19
ISIS 416859	7.8	14.0	45	16
ISIS 416860	7.8	14.1	45	17
ISIS 416861	7.7	14.6	45	15
ISIS 416863	7.6	14.1	45	17
ISIS 416866	7.8	14.0	44	20
ISIS 416867	7.8	14.0	45	14

Table 90

Effect of antisense oligonucleotide treatment on blood cell count in Sprague-Dawley rats

	Neutrophil (/mL)	Lymphocyte (/mL)	Platelets (10^3/mL)
PBS	988	7307	485
ISIS 412223	1826	16990	567
ISIS 412224	1865	16807	685
ISIS 412225	1499	13204	673
ISIS 413481	1046	12707	552
ISIS 413482	1125	11430	641
ISIS 416848	1874	14316	384
ISIS 416851	1001	9911	734
ISIS 416852	836	11956	632
ISIS 416856	3280	14328	740
ISIS 416859	1414	14323	853
ISIS 416860	1841	13986	669
ISIS 416861	1813	12865	1008
ISIS 416863	1720	14669	674

ISIS 416866	1916	16834	900
ISIS 416867	3044	10405	705

Example 40: Measurement of half-life of antisense oligonucleotide in the liver and kidney of Sprague-Dawley rats

Sprague Dawley rats were treated with ISIS antisense oligonucleotides targeting human Factor 11 and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver and kidney was evaluated.

Treatment

Groups of four Sprague Dawley rats each were injected subcutaneously twice per week for 2 weeks with 20 mg/kg of ISIS 412223, ISIS 412224, ISIS 412225, ISIS 413481, ISIS 413482, ISIS 416848, ISIS 416851, ISIS 416852, ISIS 416856, ISIS 416859, ISIS 416860, ISIS 416861, ISIS 416863, ISIS 416866, or ISIS 416867 . Three days after the last dose, the rats were sacrificed, and livers and kidneys were harvested.

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 270) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 µg/g. The results are presented in Tables 91 and 92, expressed as µg/g liver or kidney tissue. Half-lives were then calculated using WinNonlin software (PHARSIGHT).

Table 91

Full-length oligonucleotide concentration (µg/g) in the liver and kidney of Sprague-Dawley rats

ISIS No	Motif	Kidney	Liver
412223	5-10-5	551	97
412224	5-10-5	487	107

412225	5-10-5	202	119
413481	5-10-5	594	135
413482	5-10-5	241	95
416848	5-10-5	488	130
416851	5-10-5	264	193
416852	5-10-5	399	108
416856	5-10-5	378	84
416859	5-10-5	253	117
416860	5-10-5	247	94
416861	5-10-5	187	159
416863	5-10-5	239	82
416866	5-10-5	210	98
416867	5-10-5	201	112

Table 92

Total oligonucleotide concentration (μg/g) in the liver and kidney of Sprague-Dawley rats

ISIS No	Motif	Kidney	Liver
412223	5-10-5	395	86
412224	5-10-5	292	78
412225	5-10-5	189	117
413481	5-10-5	366	96
413482	5-10-5	217	91
416848	5-10-5	414	115
416851	5-10-5	204	178
416852	5-10-5	304	87
416856	5-10-5	313	80
416859	5-10-5	209	112
416860	5-10-5	151	76
416861	5-10-5	165	144
416863	5-10-5	203	79
416866	5-10-5	145	85
416867	5-10-5	157	98

Table 93

Half-life (days) of ISIS oligonucleotides in the liver and kidney of Sprague-Dawley rats

ISIS No	Motif	Half-life
412223	5-10-5	22
412224	5-10-5	17
412225	5-10-5	21
413481	5-10-5	21

413482	5-10-5	20
416848	5-10-5	24
416851	5-10-5	24
416852	5-10-5	28
416856	5-10-5	19
416859	5-10-5	19
416860	5-10-5	21
416861	5-10-5	25
416863	5-10-5	20
416866	5-10-5	16
416867	5-10-5	19

Example 41: Tolerability of antisense oligonucleotides targeting human Factor 11 in CD1 mice

ISIS oligonucleotides with 6-8-6 MOE and 5-8-5 MOE motifs targeting human Factor 11 were administered in CD1 mice evaluated for changes in the levels of various metabolic markers.

Treatment

Groups of five CD1 mice each were injected subcutaneously twice per week for 6 weeks with 50 mg/kg of ISIS 416850, ISIS 445498, ISIS 445503, ISIS 445504, ISIS 445505, ISIS 445509, ISIS 445513, ISIS 445522, ISIS 445530, ISIS 445531, or ISIS 445532. A control group of five CD1 mice was injected subcutaneously with PBS twice per week for 6 weeks. Body weight measurements were taken before and at the end of the treatment period. Three days after the last dose, the mice were sacrificed, organ weights were measured, and blood was collected for further analysis.

Body weight and organ weight

The body weight changes in the mice are presented in Table 94 and are expressed increase in grams over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 94 as percentage of the body weight. Those antisense oligonucleotides which did not affect more than six-fold increases in liver and spleen weight above the PBS control were selected for further studies.

Table 94
Change in body and organ weights of CD1 mice after antisense oligonucleotide treatment

	Body weight (g)	Liver (%)	Kidney (%)	Spleen (%)
PBS	10	5	1.6	0.3
ISIS 416850	11	6	1.5	0.4
ISIS 445498	10	6	1.6	0.5
ISIS 445503	9	8	1.4	0.6
ISIS 445504	11	6	1.6	0.4
ISIS 445505	12	6	1.5	0.5
ISIS 445509	10	6	1.6	0.5
ISIS 445513	9	5	1.6	0.4
ISIS 445522	11	6	1.7	0.4
ISIS 445530	11	6	1.5	0.5
ISIS 445531	10	6	1.5	0.5
ISIS 445532	10	6	1.6	0.4

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Measurements of alanine transaminase (ALT) and aspartate transaminase (AST) are expressed in IU/L and the results are presented in Table 95. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin and albumin were also measured and results are also presented in Table 95 and expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies.

Table 95
Effect of antisense oligonucleotide treatment on metabolic markers in the liver of CD1 mice

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)
PBS	34	49	0.23	3.6
ISIS 416850	90	115	0.20	3.2
ISIS 445498	66	102	0.24	3.4
ISIS 445503	1314	852	0.28	3.4
ISIS 445504	71	107	0.17	3.4

ISIS 445505	116	153	0.18	3.2
ISIS 445509	80	117	0.17	3.1
ISIS 445513	37	84	0.22	3.1
ISIS 445522	51	110	0.19	3.4
ISIS 445530	104	136	0.18	3.2
ISIS 445531	60	127	0.16	3.2
ISIS 445532	395	360	0.20	2.9

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 96, expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies.

Table 96

Effect of antisense oligonucleotide treatment on BUN levels (mg/dL) in the kidney of CD1 mice

	BUN
PBS	29
ISIS 416850	28
ISIS 445498	28
ISIS 445503	29
ISIS 445504	29
ISIS 445505	29
ISIS 445509	29
ISIS 445513	27
ISIS 445522	28
ISIS 445530	26
ISIS 445531	27
ISIS 445532	23

Hematology assays

Blood obtained from all mice groups were sent to Antech Diagnostics for hematocrit (HCT) measurements, as well as measurements of the various blood cells, such as WBC (neutrophils and lymphocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 97 and 98. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies.

Table 97

Effect of antisense oligonucleotide treatment on hematologic factors in CD1 mice

	RBC (10^6/mL)	Hemoglobin (g/dL)	HCT (%)	WBC (10^3/mL)
PBS	9.6	15.0	51	6
ISIS 416850	9.8	14.8	50	6
ISIS 445498	9.4	13.9	47	5
ISIS 445503	9.2	13.6	46	8
ISIS 445504	9.6	14.7	49	5
ISIS 445505	9.6	14.6	49	5
ISIS 445509	10.2	15.3	51	5
ISIS 445513,	9.8	15.0	50	7
ISIS 445522	9.7	14.6	49	5
ISIS 445530	10.0	15.1	50	7
ISIS 445531	9.4	14.5	48	9
ISIS 445532	9.7	14.8	48	7

Table 98

Effect of antisense oligonucleotide treatment on blood cell count in CD1 mice

	Neutrophil (/mL)	Lymphocyte (/mL)	Platelets (10^3/mL)
PBS	1356	4166	749
ISIS 416850	1314	4710	614
ISIS 445498	1197	3241	802
ISIS 445503	1475	6436	309
ISIS 445504	959	3578	826
ISIS 445505	818	3447	725
ISIS 445509	1104	3758	1085
ISIS 445513	959	5523	942
ISIS 445522	698	3997	1005

ISIS 445530	930	5488	849
ISIS 445531	2341	6125	996
ISIS 445532	1116	5490	689

Example 42: Tolerability of antisense oligonucleotides targeting human Factor 11 in Sprague-Dawley rats

Eight antisense oligonucleotides which had been evaluated in CD1 mice (Example 41) were further evaluated in Sprague-Dawley rats for changes in the levels of various metabolic markers.

Treatment

Groups of four Sprague Dawley rats each were injected subcutaneously twice per week for 6 weeks with 50 mg/kg of ISIS 445498, ISIS 445504, ISIS 445505, ISIS 445509, ISIS 445513, ISIS 445522, ISIS 445530, or ISIS 445531. A control group of Sprague Dawley rats was injected subcutaneously with PBS twice per week for 6 weeks. Body weight measurements were taken before and throughout the treatment period. Three days after the last dose, urine samples were collected and the rats were then sacrificed, organ weights were measured, and blood was collected for further analysis.

Body weight and organ weight

The body weights of the rats were measured at the onset of the study and subsequently twice per week. The body weights are presented in Table 99 and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 99 as a percentage of the body weight. Those antisense oligonucleotides which did not affect more than six-fold increases in liver and spleen weight above the PBS control were selected for further studies.

Table 99
Change in body and organ weights of Sprague Dawley rats after antisense oligonucleotide treatment (%)

	Body weight	Liver	Spleen	Kidney
ISIS 445498	-17	+26	+107	-10
ISIS 445504	-15	+22	+116	+6
ISIS 445505	-21	+12	+146	+2
ISIS 445509	-17	+16	+252	+3

ISIS 445513	-13	+25	+194	+15
ISIS 445522	-13	+26	+184	+19
ISIS 445530	-7	+24	+99	+4
ISIS 445531	-10	+17	+89	+4

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 100 expressed in IU/L. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer; results are presented in Table 100 and expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies.

Table 100

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of Sprague-Dawley rats

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)
PBS	102	36	0.13	3.7
ISIS 445498	417	124	0.14	3.7
ISIS 445504	206	86	0.11	3.5
ISIS 445505	356	243	0.15	3.6
ISIS 445509	676	291	0.14	3.5
ISIS 445513	214	91	0.15	3.5
ISIS 445522	240	138	0.47	3.6
ISIS 445530	116	56	0.11	3.7
ISIS 445531	272	137	0.12	3.7

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 101, expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies. The total urine protein and ratio of urine protein to creatinine in total urine samples after antisense oligonucleotide treatment was calculated and is also presented in Table 101. Those antisense oligonucleotides which did not affect more than a five-fold increase in urine protein/creatinine ratios compared to the PBS control were selected for further studies.

Table 101

Effect of antisense oligonucleotide treatment on metabolic markers in the kidney of Sprague-Dawley rats

	BUN (mg/dL)	Creatinine (mg/dL)	Urine protein/creatinine ratio
PBS	18	0.4	1.4
ISIS 445498	25	0.5	3.1
ISIS 445504	26	0.4	4.3
ISIS 445505	24	0.4	3.8
ISIS 445509	27	0.5	4.0
ISIS 445513	24	0.4	4.6
ISIS 445522	25	0.4	6.4
ISIS 445530	22	0.4	4.2
ISIS 445531	23	0.4	3.4

Hematology assays

Blood obtained from all rat groups were sent to Antech Diagnostics for hematocrit (HCT) measurements, as well as measurements of the various blood cells, such as WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 102 and 103. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies.

Table 102

Effect of antisense oligonucleotide treatment on hematologic factors in Sprague-Dawley rats

	RBC (/pL)	Hemoglobin (g/dL)	HCT (%)	WBC (/nL)
PBS	8.8	16.0	55	13
ISIS 445498	8.5	14.7	49	13
ISIS 445504	8.9	14.7	50	16
ISIS 445505	9.1	15.0	50	21
ISIS 445509	8.4	14.1	47	17
ISIS 445513	7.8	13.0	44	17
ISIS 445522	7.7	13.6	47	18
ISIS 445530	8.9	14.7	50	12
ISIS 445531	8.8	14.8	50	13

Table 103
Effect of antisense oligonucleotide treatment on blood cell count in Sprague-Dawley rats

	Neutrophil (%)	Lymphocyte (%)	Monocytes (%)	Platelets (/nL)
PBS	14	82	2.0	1007
ISIS 445498	9	89	2.0	1061
ISIS 445504	10	87	2.0	776
ISIS 445505	10	87	2.5	1089
ISIS 445509	11	84	3.8	1115
ISIS 445513	14	82	3.5	1051
ISIS 445522	13	84	2.8	1334
ISIS 445530	11	87	2.0	1249
ISIS 445531	10	86	2.8	1023

Example 43: Tolerability of antisense oligonucleotides targeting human Factor 11 in CD1 mice

ISIS oligonucleotides with 4-8-4 MOE, 3-8-3 MOE, 2-10-2 MOE, 3-10-3 MOE, and 4-10-4 MOE motifs targeting human Factor 11 were administered in CD1 mice evaluated for changes in the levels of various metabolic markers.

Treatment

Groups of five CD1 mice each were injected subcutaneously twice per week for 6 weeks with 50 mg/kg of ISIS 449707, ISIS 449708, ISIS 449409, ISIS 449710, or ISIS 449711. A control group of five CD1 mice was injected subcutaneously with PBS twice per week for 6 weeks. Body weight measurements were taken before and at the end of the treatment period. Three days after the last dose, the mice were sacrificed, organ weights were measured, and blood was collected for further analysis.

Body weight and organ weight

The body weights of the mice taken at the end of the study are presented in Table 104 and are expressed in grams. Liver, spleen and kidney weights were also measured at the end of the study and are also presented in Table 104 as percentage of the body weight. Those antisense oligonucleotides which did not affect more than six-fold increases in liver and spleen weight above the PBS control were selected for further studies.

Table 104
Change in body and organ weights of CD1 mice after antisense oligonucleotide treatment

	Body weight (g)	Liver (%)	Spleen (%)	Kidney (%)
PBS	39	-	-	-
ISIS 449707	42	+11	+63	-5
ISIS 449708	40	+17	+66	0
ISIS 449709	40	+15	+62	-14
ISIS 449710	42	+6	+43	-7
ISIS 449711	42	+18	+63	-12

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 105 expressed in IU/L. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer and results are presented in Table 105 and expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies.

Table 105
Effect of antisense oligonucleotide treatment on metabolic markers in the liver of CD1 mice

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)
PBS	39	52	0.22	3.2
ISIS 449707	41	62	0.19	2.3
ISIS 449708	66	103	0.17	2.8
ISIS 449709	62	83	0.18	2.8
ISIS 449710	43	95	0.18	2.8
ISIS 449711	52	83	0.22	2.8

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 106, expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies.

Table 106

Effect of antisense oligonucleotide treatment on metabolic markers (mg/dL) in the kidney of CD1 mice

	BUN	Creatinine
PBS	28	0.3
ISIS 449707	27	0.2
ISIS 449708	28	0.2
ISIS 449709	34	0.3
ISIS 449710	29	0.2
ISIS 449711	26	0.2

Hematology assays

Blood obtained from all mice groups were sent to Antech Diagnostics for hematocrit (HCT), measurements, as well as measurements of the various blood cells, such as WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 107 and 108. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies.

Table 107

Effect of antisense oligonucleotide treatment on hematologic factors in CD1 mice

	RBC (/pL)	Hemoglobin (g/dL)	Hematocrit (%)	WBC (/nL)
PBS	9.8	14.6	54	6
ISIS 449707	8.4	12.4	45	6
ISIS 449708	9.2	13.2	48	7
ISIS 449709	9.2	13.2	49	5
ISIS 449710	9.1	13.5	48	7
ISIS 449711	9.0	13.3	48	6

Table 108

Effect of antisense oligonucleotide treatment on blood cell count in CD1 mice

	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Platelets (/nL)
PBS	15	80	3	1383
ISIS 449707	11	85	3	1386
ISIS 449708	17	77	5	1395

ISIS 449709	19	76	4	1447
ISIS 449710	15	81	3	1245
ISIS 449711	15	79	6	1225

Example 44: Tolerability of antisense oligonucleotides targeting human Factor 11 in Sprague-Dawley rats

Five antisense oligonucleotides which had been evaluated in CD1 mice (Example 43) were further evaluated in Sprague-Dawley rats for changes in the levels of various metabolic markers.

Treatment

Groups of four Sprague Dawley rats each were injected subcutaneously twice per week for 6 weeks with 50 mg/kg of ISIS 449707, ISIS 449708, ISIS 449709, ISIS 449710, or ISIS 449711. A control group of four Sprague Dawley rats was injected subcutaneously with PBS twice per week for 6 weeks. Body weight measurements were taken before and throughout the treatment period. Three days after the last dose, urine samples were collected and the rats were then sacrificed, organ weights were measured, and blood was collected for further analysis.

Body weight and organ weight

The body weights of the rats were measured at the onset of the study and at the end of the study. The body weight changes are presented in Table 109 and are expressed as increase in grams over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 109 as a percentage of the body weight. Those antisense oligonucleotides which did not affect more than six-fold increases in liver and spleen weight above the PBS control were selected for further studies.

Table 109

Change in body and organ weights of Sprague Dawley rats after antisense oligonucleotide treatment

	Body weight (g)	Liver (%)	Spleen (%)	Kidney (%)
PBS	478	-	-	-
ISIS 449707	352	+41	+400	+80
ISIS 449708	382	+31	+259	+40
ISIS 449709	376	+8	+231	+19
ISIS 449710	344	+82	+302	+50
ISIS 449711	362	+52	+327	+72

Liver function

To evaluate the impact of ISIS oligonucleotides on hepatic function, plasma concentrations of ALT and AST were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of alanine transaminase (ALT) and aspartate transaminase (AST) were measured and the results are presented in Table 110 expressed in IU/L. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin and albumin were also measured and results are presented in Table 110 and expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies.

Table 110

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of Sprague-Dawley rats

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)
PBS	41	107	0.1	3.4
ISIS 449707	61	199	0.2	3.1
ISIS 449708	25	90	0.1	3.2
ISIS 449709	63	126	0.2	3.1
ISIS 449710	36	211	0.1	2.9
ISIS 449711	32	163	0.1	2.9

Kidney function

To evaluate the impact of ISIS oligonucleotides on kidney function, plasma concentrations of BUN and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 111, expressed in mg/dL. Those antisense oligonucleotides which did not affect more than a two-fold increase in BUN levels compared to the PBS control were selected for further studies. The total urine protein and ratio of urine protein to creatinine in total urine samples after antisense oligonucleotide treatment was calculated and is also presented in Table 111. Those antisense oligonucleotides which did not affect more than a five-fold increase in urine protein/creatinine ratios compared to the PBS control were selected for further studies.

Table 111
Effect of antisense oligonucleotide treatment on metabolic markers in the kidney of Sprague-Dawley rats

	BUN (mg/dL)	Creatinine (mg/dL)	Urine protein/creatinine ratio
PBS	22	0.4	1.5
ISIS 449707	24	0.4	3.2
ISIS 449708	24	0.4	5.7
ISIS 449709	24	0.4	3.4
ISIS 449710	29	0.3	5.9
ISIS 449711	28	0.4	7.3

Hematology assays

Blood obtained from all rat groups were sent to Antech Diagnostics for hematocrit (HCT) measurements, as well as measurements of the various blood cells, such as WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 112 and 113. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies.

Table 112
Effect of antisense oligonucleotide treatment on hematologic factors in Sprague-Dawley rats

	RBC (/pL)	Hemoglobin (g/dL)	Hematocrit (%)	WBC (/nL)
PBS	8.2	15.1	50	16
ISIS 449707	6.0	12.0	40	20
ISIS 449708	6.6	12.2	40	22
ISIS 449709	6.9	12.6	41	14
ISIS 449710	6.3	12.5	41	13
ISIS 449711	6.4	12.6	43	13

Table 113
Effect of antisense oligonucleotide treatment on blood cell count in Sprague-Dawley rats

	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Platelets (/nL)
PBS	12	84	2	1004
ISIS 449707	6	91	2	722
ISIS 449708	6	92	2	925
ISIS 449709	5	91	3	631
ISIS 449710	6	91	2	509
ISIS 449711	7	90	2	919

Example 45: Dose-dependent pharmacologic effect of antisense oligonucleotides targeting human Factor 11 in cynomolgus monkeys

Several antisense oligonucleotides were tested in cynomolgus monkeys to determine the pharmacologic effects of the oligonucleotides on Factor 11 activity, anticoagulation and bleeding times, liver and kidney distributions, and tolerability. All the ISIS oligonucleotides used in this study target human Factor 11 mRNA and are also fully cross-reactive with the rhesus monkey gene sequence (see Table 44). It is expected that the rhesus monkey ISIS oligonucleotides are fully cross-reactive with the cynomolgus monkey gene sequence as well. At the time the study was undertaken, the cynomolgus monkey genomic sequence was not available in the National Center for Biotechnology Information (NCBI) database; therefore, cross-reactivity with the cynomolgus monkey gene sequence could not be confirmed.

Treatment

Groups, each consisting of two male and three female monkeys, were injected subcutaneously with ISIS 416838, ISIS 416850, ISIS 416858, ISIS 416864, or ISIS 417002 in escalating doses. Antisense oligonucleotide was administered to the monkeys at 5 mg/kg three times per a week for week 1; 5 mg/kg twice per week for weeks 2 and 3; 10 mg/kg three times per week for week 4; 10 mg/kg twice per week for weeks 5 and 6; 25 mg/kg three times per week for week 7; and 25 mg/kg twice per week for weeks 8, 9, 10, 11, and 12. One control group, consisting of two male and three female monkeys, was injected subcutaneously with PBS according to the same dosing regimen. An additional experimental group, consisting of two male and three female monkeys, was injected subcutaneously with ISIS 416850 in a chronic, lower dose regimen. Antisense oligonucleotide was administered to the monkeys at 5 mg/kg three times per week for week 1; 5 mg/kg twice per week for week 2 and 3; 10 mg/kg three times per week for week 4; and 10 mg/kg twice per week for weeks 5 to 12. Body weights were measured weekly. Blood samples were collected 14 days and 5 days before the start of treatment and subsequently once per week for Factor 11 protein activity analysis in plasma, fibrinogen measurement, PT and aPTT measurements, bleeding times, and measurement of various hematologic factors. On day 85, the monkeys were euthanized by exsanguination while under deep anesthesia, and organs harvested for further analysis.

RNA analysis

On day 85, RNA was extracted from liver tissue for real-time PCR analysis of Factor 11 using primer probe set LTS00301 (forward primer sequence ACACGCATTAAAAAGAGCAAAGC, designated herein as SEQ ID NO 271; reverse primer sequence CAGTGTCATGGTAAAATGAAGAATGG, designated herein as SEQ ID NO: 272; and probe sequence TGCAGGCACAGCATCCCAGTGTTCTX, designated herein as SEQ ID NO. 273). Results are presented as percent inhibition of Factor 11, relative to PBS control. As shown in Table 114, treatment with ISIS oligonucleotides resulted in significant reduction of Factor 11 mRNA in comparison to the PBS control.

Table 114

Inhibition of Factor 11 mRNA in the cynomolgus monkey liver relative to the PBS control

ISIS No	% inhibition
416838	37
416850	84
416858	90
416864	44
417002	57

Protein analysis

Plasma samples from all monkey groups taken on different days were analyzed by a sandwich-style ELISA assay (Affinity Biologicals Inc.) using an affinity-purified polyclonal anti-Factor 11 antibody as the capture antibody and a peroxidase-conjugated polyclonal anti-Factor 11 antibody as the detecting antibody. Monkey plasma was diluted 1:50 for the assay. Peroxidase activity was expressed by incubation with the substrate o-phenylenediamine. The color produced was quantified using a microplate reader at 490 nm and was considered to be proportional to the concentration of Factor 11 in the samples.

The results are presented in Table 115, expressed as percentage reduction relative to that of the PBS control. Treatment with ISIS 416850 and ISIS 416858 resulted in a time-dependent decrease in protein levels.

Table 115

Inhibition of Factor 11 protein in the cynomolgus monkey liver relative to the PBS control

Days	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	0	0	0	0	0	0
-5	0	0	0	5	0	1
8	3	8	6	7	0	6
15	4	4	16	9	4	13
22	5	11	23	7	2	12
29	8	15	28	10	8	20
36	11	17	35	9	8	22
43	5	23	39	9	9	24
50	8	42	49	10	13	30
57	10	49	60	7	24	34
64	11	55	68	5	26	37
71	12	57	71	10	30	41
78	10	63	73	9	22	42
85	10	64	78	8	23	34

PT and aPTT assay

Blood samples were collected in tubes containing sodium citrate. PT and aPTT were determined in duplicate with an ACL 9000 coagulation instrument (Instrumentation Laboratory, Italy). The results were interpolated on a standard curve of serial dilutions citrated control monkey plasma tested to give a reported result in percent normal.

Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) were measured using platelet poor plasma (PPP) from monkeys treated with ISIS oligonucleotides. PT and aPTT values are provided in Tables 116 and 117 and are reported as International Normalized Ratio (INR) values. INR values for PT and aPTT were determined by dividing the PT or aPTT value for each experimental group by the PT or aPTT for the PBS treated group. This ratio was then raised to the power of the International Sensitivity Index (ISI) of the tissue factor used. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

As shown in Table 116, PT was not significantly prolonged in monkeys treated with ISIS oligonucleotides either in the escalating dose regimen or the chronic dose regimen. However, aPTT was prolonged in a dose-dependent manner, as presented in Table 117. These data suggest that antisense reduction of Factor 11 affects the contact activation pathway, but not the extrinsic pathway of blood coagulation. Therefore, antisense reduction of Factor 11 is useful for inhibiting the formation of a thrombus or clot in response to an abnormal vessel wall, but not in response to tissue injury.

Table 116
Effect of ISIS antisense oligonucleotides on PT ratio in cynomolgus monkeys

day	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	1.00	1.00	1.00	1.00	1.00	1.00
-5	1.00	1.00	1.00	1.00	1.00	1.00
8	1.03	1.00	1.05	1.02	1.02	1.03
15	1.03	1.02	1.07	1.07	1.04	1.06
22	1.07	1.02	1.06	1.03	1.04	1.06
29	1.03	1.03	1.08	1.06	1.01	1.00
36	1.05	1.02	1.07	1.06	1.05	1.06
43	1.03	1.01	1.08	1.04	1.03	1.02
50	1.02	1.02	1.03	1.01	0.99	0.98
57	1.04	1.04	1.09	1.08	1.03	n.d.
64	1.04	1.03	1.09	1.10	1.03	n.d.
71	1.02	1.03	1.07	1.07	0.99	n.d.
78	1.04	1.05	1.10	1.08	1.02	n.d.
85	1.05	1.04	1.07	1.13	1.02	n.d.

n.d.=no data

Table 117
Effect of ISIS antisense oligonucleotides on aPTT ratio in cynomolgus monkeys

day	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	1.00	1.00	1.00	1.00	1.00	1.00
-5	1.00	1.00	1.00	1.00	1.00	1.00
8	1.07	1.05	1.03	1.05	1.05	1.12
15	1.05	1.05	1.07	1.03	1.03	1.07
22	1.20	1.13	1.18	1.11	1.16	1.21
29	1.19	1.13	1.20	1.13	1.11	1.26
36	1.20	1.26	1.36	1.19	1.18	1.34
43	1.18	1.17	1.28	1.07	1.06	1.22
50	1.25	1.68	1.55	1.26	1.18	1.35
57	1.21	1.59	1.59	1.19	1.22	n.d.
64	1.18	1.64	1.60	1.12	1.11	n.d.
71	1.15	1.76	1.70	1.18	1.16	n.d.

78	1.19	1.88	1.79	1.18	1.18	n.d.
85	1.22	1.99	1.76	1.25	1.20	n.d.

n.d.=no data

Protein activity analysis

Blood samples were collected at various time points and Factor 11 proenzyme was measured using a F11 assay based on clotting time. Clotting times were determined in duplicate with a ST4 semi-automated coagulation instrument (Diagnostica Stago, NJ). Thirty µl of citrated sample plasma diluted 1/20 in HEPES-NaCl buffer with BSA was incubated with 30µl aPTT reagent (Automated aPTT, Organon Technika, NC) and 30µl of citrated plasma deficient of Factor 11 (George King Bio-Medical Inc.) at 37°C for 5 min, followed by the addition of 30 µl of 25 mM $CaCl_2$ to initiate clotting. Results were interpolated on a standard curve of serially diluted citrated control plasma.

Results are presented in Table 118 as percent inhibition of Factor 11 activity, relative to PBS control. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

Table 118

Inhibition of Factor 11 protein by ISIS antisense oligonucleotides given in escalating dose/chronic dose regimen in cynomolgus monkeys

Days before/after treatment	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	0	0	0	0	0	0
-5	0	0	0	5	0	1
8	3	8	6	7	0	6
15	4	4	16	9	4	13
22	5	11	23	7	2	12
29	8	15	28	10	8	20
36	11	17	35	9	8	24
43	5	23	39	9	9	24
50	8	42	49	10	13	30
57	10	49	60	7	24	n.d.
64	11	55	68	5	26	n.d.
71	12	57	71	10	30	n.d.
78	10	63	73	9	22	n.d.
85	10	64	78	8	23	n.d.

n.d.=no data

Fibrinogen assay

Nine parts of fresh monkey plasma was collected into one part of trisodium citrate. The samples were evaluated of fibrinogen content using an ACL 9000 coagulation instrument (Instrumentation Laboratory, Italy). Results are presented in Table 119 expressed in mg/dL. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

Table 119

Effect of ISIS antisense oligonucleotides on fibrinogen levels in cynomolgus monkeys

Days before/after treatment	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	296	251	310	277	300	291	274
-5	246	205	261	246	243	222	227
8	245	209	281	246	227	221	232
15	207	198	270	219	210	195	174
22	219	183	243	222	184	199	192
29	231	184	234	220	205	199	192
36	235	182	232	225	202	191	185
43	231	186	219	229	198	187	194
50	251	216	215	259	233	236	204
57	235	190	186	225	200	201	n.d.
64	240	190	190	236	218	236	n.d.
71	233	199	178	239	245	228	n.d.
78	234	189	177	234	250	221	n.d.
85	246	196	187	243	240	224	n.d.

n.d.=no data

Bleeding Assay

On different days during the treatment period, bleeding assay was performed using a Surgicutt Jr. device (ITC, New Jersey). Monkeys were placed in monkey chair with their arm placed in a steady support. The arm was lightly shaved and a sphygmomanometer was placed on the upper arm. The cuff of the sphygmomanometer was inflated to 40 mm Hg and this pressure was maintained throughout the procedure. The area on the upper arm to be incised was cleansed with an antiseptic swab and the Surgicutt Jr device was used to make an incision over the lateral aspect, volar surface of the forearm, parallel to and 5 cm below the antecubital crease. At the exact moment the incision was made, a stopwatch was started. Every 30 seconds, blood from the incision was blotted out using a blotting paper without directly touching the incision, so that formation of the platelet plug was not disturbed. Blood was blotted out every 30 seconds until blood no longer stained the paper. The stopwatch was then stopped and the bleeding time determined. The sphygmomanometer was removed from the animal's arm, the incision site was antiseptically swabbed and a wound closure strip applied. The results are provided in Table X, expressed in seconds. The results are provided in Table 120. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

These data suggest that the hemorrhagic potential of the compounds provided herein is low.

Table 120
Bleeding assay in cynomolgus monkeys

Days before/after treatment	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	147	200	172	154	166	185	177
-5	153	150	127	149	111	175	93
15	111	167	165	146	153	174	149
22	113	165	151	100	133	194	143
36	174	166	137	206	205	186	221
43	157	120	216	111	146	120	156
57	147	238	195	138	216	206	n.d.
64	113	131	201	113	218	146	n.d.
78	114	145	203	186	170	163	n.d.
85	147	201	201	191	203	182	n.d.

Platelet aggregation assay

Platelet aggregation was initiated by adding 1 mmol/L ADP and/or 3 μg collagen (depending on the collection day, as outlined in Table 121) to plasma samples, and was allowed to proceed for 10 minutes. Aggregation was characterized by recording the change in the electrical resistance or impedance and the change in the initial slope of aggregation after platelet shape change. The aggregation test was performed twice per sample on each collection day and the average value was taken. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

Table 121
Effect of antisense oligonucleotide treatment on platelet aggregation in cynomolgus monkeys in Ohms

	day -5 (with collagen)	day 15 (with ADP)	day 36 (with ADP)	day 43 (with collagen)	day 57 (with ADP)	day 64 (with collagen)	day 78 (with ADP)	day 85 (with ADP)	day 85 (with collagen)
PBS	17	15	7	14	16	13	12	16	17
ISIS 416838	15	15	8	16	7	13	11	15	24
ISIS 416850	23	12	16	16	18	17	9	22	26
ISIS 416858	22	19	17	16	11	14	8	18	23
ISIS 416864	27	20	17.8	20	18	17	13	22	28
ISIS 417002	21	16	13.9	19	18	18	18	22	24
ISIS 416850*	21	14	11.6	21	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.=no data

Body and organ weights

Body weights were taken once weekly throughout the dosing regimen. The measurements of each group are given in Table 122 expressed in grams. The results indicate that treatment with the antisense oligonucleotides did not cause any adverse changes in the health of the animals, which may have resulted in a significant alteration in weight compared to the PBS control. Organ weights were taken after the animals were euthanized and livers, kidneys and spleens were harvested and weighed. The results are presented in Table 123 and also show no significant alteration in weights compared to the PBS control, except for ISIS 416858, which shows increase in spleen weight. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

Table 122
Weekly measurements of body weights (g) of cynomolgus monkeys

day	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
1	2780	2720	2572	2912	2890	2640	2665
8	2615	2592	2430	2740	2784	2523	2579
15	2678	2642	2474	2760	2817	2571	2607
22	2715	2702	2514	2800	2857	2617	2661

29	2717	2689	2515	2763	2863	2622	2667
36	2738	2708	2545	2584	3327	2631	2656
43	2742	2700	2544	2607	3355	2630	2670
50	2764	2731	2613	2646	3408	2652	2679
57	2763	2737	2629	2617	3387	2654	n.d.
64	2781	2746	2642	2618	3384	2598	n.d.
71	2945	2869	2769	2865	2942	2727	n.d.
78	2815	2766	2660	2713	2822	2570	n.d.

n.d.=no data

Table 123

Organ weights (g) of cynomolgus monkeys after antisense oligonucleotide treatment

	Liver	Spleen	Kidney
PBS	46	4	11
ISIS 416838	63	5	12
ISIS 416580	64	4	16
ISIS 416858	60	12	13
ISIS 416864	53	5	14
ISIS 417002	51	5	15

Liver function

To evaluate the impact of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Tables 124 and 125 expressed in IU/L. Those antisense oligonucleotides which did not affect an increase in ALT/AST levels above seven-fold of control levels were selected for further studies. Plasma levels of bilirubin were also measured and results are presented in Table 126 expressed in mg/dL. Those antisense oligonucleotides which did not affect an increase in levels of bilirubin more than two-fold of the control levels by antisense oligonucleotide treatment were selected for further studies. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

Table 124
Effect of antisense oligonucleotide treatment on ALT (IU/L) in the liver of cynomolgus monkeys

Days before/after treatment	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	57	76	54	47	54	61	80
22	39	36	41	28	37	36	42
43	36	35	43	36	36	35	41
64	38	40	60	47	43	42	n.d.
85	34	41	75	50	43	116	n.d.

n.d.=no data

Table 125
Effect of antisense oligonucleotide treatment on AST (IU/L) in the liver of cynomolgus monkeys

Days before/after treatment	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	71	139	81	58	76	114	100
22	43	39	45	38	41	44	39
43	38	32	50	39	40	42	40
64	35	33	56	50	46	37	n.d.
85	41	30	82.	49	56	50	n.d.

n.d.=no data

Table 126
Effect of antisense oligonucleotide treatment on bilirubin (mg/dL) in the liver of cynomolgus monkeys

Days before/after treatment	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
-14	0.24	0.26	0.21	0.27	0.31	0.26	0.28
22	0.16	0.17	0.13	0.18	0.22	0.20	0.19
43	0.17	0.17	0.13	0.14	0.17	0.21	0.18
64	0.19	0.15	0.14	0.12	0.16	0.14	n.d.
85	0.20	0.13	0.14	0.14	0.17	0.12	n.d.

n.d.=no data

Kidney function

To evaluate the impact of ISIS oligonucleotides on kidney function, urine samples were collected. The ratio of urine protein to creatinine in urine samples after antisense oligonucleotide treatment was calculated and is presented in Table 127. Those antisense oligonucleotides which did not affect more than a five-fold increase in urine protein/creatinine ratios compared to the PBS control were selected for further studies.

Table 127

Effect of antisense oligonucleotide treatment on urine protein to creatinine ratio in cynomolgus monkeys

	Day 80	Day 84
PBS	0.09	0.10
ISIS 416838	0.13	0.13
ISIS 416850	0.09	0.12
ISIS 416858	0.10	0.07
ISIS 416864	0.36	0.34
ISIS 417002	0.18	0.24

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide as well as the elapsed time oligonucleotide degradation and elimination from the liver and kidney were evaluated. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 270) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. Half-lives were then calculated using WinNonlin software (PHARSIGHT). The results are presented in Tables 128 and 129, expressed as μg/g liver or kidney tissue.

Table 128

Full-length oligonucleotide concentration (μg/g) in the liver and kidney of cynomolgus monkeys

ISIS No.	Kidney	Liver
416838	1339	1087
416850	2845	1225
416858	1772	1061
416864	2093	1275
417002	2162	1248

Table 129
Total oligonucleotide concentration (μg/g) in the liver and kidney of cynomolgus monkeys

ISIS No.	Kidney	Liver
416838	1980	1544
416850	3988	1558
416858	2483	1504
416864	3522	1967
417002	3462	1757

Hematology assays

Blood obtained from all monkey groups were sent to Korea Institute of Toxicology (KIT) for HCT, MCV, MCH, and MCHC analysis, as well as measurements of the various blood cells, such as WBC (neutrophils, lymphocytes, monocytes, eosinophils, basophils, reticulocytes), RBC, platelets and total hemoglobin content. The results are presented in Tables 130-143. Those antisense oligonucleotides which did not affect a decrease in platelet count of more than 50% and an increase in monocyte count of more than three-fold were selected for further studies. The ISIS oligonucleotide, ISIS 416850, given with the chronic dose regimen is distinguished from the other oligonucleotides with an asterisk (*).

Table 130
Effect of antisense oligonucleotide treatment on WBC count (x10^3/μL) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	14	12	13	14	13	13	15
day -5	13	12	13	14	13	14	15
day 8	10	10	10	12	11	10	13
day 15	10	10	9	11	10	10	16
day 22	12	11	10	11	10	10	15
day 29	11	11	11	12	10	10	14
day 36	10	10	10	12	10	11	16
day 43	10	10	9	11	10	10	15
day 50	12	11	11	13	12	13	15
day 57	11	12	11	13	12	12	n.d.
day 64	11	13	11	12	11	11	n.d.
day 71	15	15	15	13	14	12	n.d.
day 78	10	11	12	11	11	9	n.d.
day 85	10	12	15	11	12	10	n.d.

n.d.=no data

Table 131
Effect of antisense oligonucleotide treatment on RBC count (x10^6/μL) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	5.7	5.6	5.3	5.6	5.5	5.6	5.5
day -5	5.7	5.6	5.5	5.6	5.6	5.6	5.5
day 8	5.7	5.7	5.4	5.6	5.7	5.6	5.5
day 15	5.6	5.6	5.3	5.4	5.7	5.4	5.3
day 22	5.5	5.4	5	5.3	5.3	5.2	5.1
day 29	5.6	5.3	4.9	5.3	5.3	5.2	5.2
day 36	5.7	5.5	5.3	5.5	5.6	5.4	5.3
day 43	5.7	5.6	5.2	5.5	5.5	5.4	5.2
day 50	5.8	5.5	5.2	5.5	5.6	5.4	5.3
day 57	5.7	5.5	5.2	5.6	5.5	4.9	n.d.
day 64	5.8	5.6	5.4	5.7	5.6	5.4	n.d.
day 71	5.6	5.5	5.4	5.6	5.6	5.5	n.d.
day 78	5.6	5.4	5.3	5.4	5.3	5.4	n.d.
day 85	5.6	5.5	5.5	5.5	5.4	5.4	n.d.

n.d.=no data

Table 132
Effect of antisense oligonucleotide treatment on hemoglobin (g/dL) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	13.2	12.9	12.4	13.2	12.7	13.0	12.8
day -5	13.1	13.1	12.7	13.2	13.0	13.2	12.8
day 8	13.1	12.9	12.4	12.8	12.7	12.8	12.5
day 15	12.9	12.9	12.1	12.6	12.8	12.3	12.2
day 22	12.7	12.5	11.6	12.4	12.1	12.1	11.7
day 29	12.8	12.4	11.5	12.3	12.1	12.0	12.0
day 36	13.0	12.8	12.2	12.6	12.5	12.5	12.3
day 43	12.9	12.7	11.8	12.4	12.2	12.3	11.8
day 50	12.6	12.3	11.8	12.2	12.1	12.3	11.9
day 57	13.1	12.6	12.1	12.7	12.3	11.3	n.d.
day 64	13.1	12.6	12.3	12.8	12.1	12.2	n.d.
day 71	12.9	12.7	12.3	12.7	12.2	12.5	n.d.
day 78	13.0	12.5	12.2	12.4	11.9	12.4	n.d.
day 85	13.2	12.4	12.7	11.9	12.3	12.2	n.d.

n.d.=no data

Table 133
Effect of antisense oligonucleotide treatment on hematocrit (%) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	46	42	41	43	43	44	44
day -5	44	42	43	42	44	45	43
day 8	44	43	43	43	44	44	43
day 15	44	42	40	40	42	40	40
day 22	45	43	41	41	42	41	40
day 29	46	43	41	41	43	42	42
day 36	46	43	42	40	42	42	41
day 43	46	43	40	40	42	41	40
day 50	48	44	42	41	44	43	42
day 57	46	43	42	41	42	38	n.d.
day 64	47	44	43	42	42	41	n.d.
day 71	46	44	43	42	44	43	n.d.
day 78	43	41	41	39	39	40	n.d.
day 85	43	42	42	39	40	41	n.d.

n.d.=no data

Table 134
Effect of antisense oligonucleotide treatment on MCV (fL) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	81	77	78	77	79	79	81
day -5	78	76	77	75	79	80	78
day 8	77	77	80	77	78	79	79
day 15	78	75	76	74	74	76	75
day 22	84	80	83	77	79	79	79
day 29	83	81	83	78	80	81	82
day 36	81	78	80	75	76	78	76
day 43	80	78	79	74	77	77	77
day 50	84	80	83	76	79	80	80
day 57	82	79	80	74	77	80	n.d.
day 64	81	79	79	73	75	76	n.d.
day 71	84	80	80	75	79	78	n.d.
day 78	78	76	79	72	74	75	n.d.
day 85	77	77	77	72	74	76	n.d.

n.d.=no data

Table 135
Effect of antisense oligonucleotide treatment on MCH (pg) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	23	23	23	24	23	24	24
day -5	23	23	23	23	23	24	23
day 8	23	23	23	23	23	23	23
day 15	23	23	23	23	23	23	23
day 22	23	23	24	24	23	23	23
day 29	23	23	23	23	23	23	23
day 36	23	23	23	23	23	23	23
day 43	23	23	23	23	22	23	23
day 50	22	23	23	23	22	23	23
day 57	23	23	23	22	23	23	n.d.
Day 64	23	23	22	22	23	22	n.d.
Day 71	23	23	23	22	23	23	n.d.
Day 78	23	23	23	23	23	23	n.d.
Day 85	23	23	22	22	23	23	n.d.

n.d.=no data

Table 136
Effect of antisense oligonucleotide treatment on MCHC (g/dL) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	29	30	30	31	29	30	29
day -5	30	31	30	31	29	30	30
day 8	30	30	29	30	29	29	29
day 15	30	31	30	31	30	31	30
day 22	28	29	28	30	29	29	29
day 29	28	29	28	30	29	29	28
day 36	28	30	29	31	30	30	30
day 43	28	30	29	31	29	30	30
day 50	26	28	28	30	28	29	29
day 57	29	29	29	31	29	29	n.d.
day 64	28	29	29	30	29	30	n.d.
day 71	28	29	28	30	28	29	n.d.
day 78	30	30	29	32	30	31	n.d.
day 85	31	30	30	31	30	30	n.d.

n.d.=no data

Table 137
Effect of antisense oligonucleotide treatment on platelet count (x $10^3/\mu L$) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	349	377	528	419	434	442	387
day -5	405	425	573	463	456	466	434
day 8	365	387	548	391	438	435	401
day 15	375	387	559	400	439	410	396
day 22	294	319	466	316	364	377	347
day 29	311	337	475	336	397	410	370
day 36	326	370	505	371	428	415	379
day 43	336	365	490	342	351	393	391
day 50	379	372	487	331	419	389	351
day 57	345	371	528	333	409	403	n.d.
day 64	329	358	496	295	383	436	n.d.
day 71	322	365	465	286	394	490	n.d.
day 78	309	348	449	262	366	432	n.d.
day 85	356	344	458	267	387	418	n.d.

n.d.=no data

Table 138
Effect of antisense oligonucleotide treatment on reticulocytes (%) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	1.4	1.0	1.7	1.0	0.9	0.9	1.1
day -5	1.0	0.9	1.2	0.9	0.9	0.8	0.8
day 8	1.0	1.2	1.2	1.2	0.8	1.1	1.1
day 15	1.5	1.2	1.9	1.6	0.8	1.1	1.0
day 22	1.2	1.2	1.9	1.3	0.9	1.2	1.0
day 29	1.6	1.6	2.5	1.5	1.3	1.6	1.4
day 36	1.7	1.6	2.2	1.6	1.3	1.3	1.3
day 43	1.3	1.2	1.6	1.3	1.1	1.1	1.0
day 50	1.6	1.6	2.7	1.5	1.3	1.6	1.2
day 57	1.8	1.5	2.0	1.4	1.0	4.6	n.d.
day 64	1.3	1.3	1.7	1.0	0.8	1.3	n.d.
day 71	1.6	1.3	1.8	1.3	1.0	1.3	n.d.
day 78	1.5	1.4	1.8	1.2	1.2	1.3	n.d.
day 85	1.5	1.5	2.3	1.3	1.5	1.4	n.d.

n.d.=no data

Table 139
Effect of antisense oligonucleotide treatment on neutrophils (%) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	40	36	49	37	53	43	48
day -5	37	35	52	46	51	43	53
day 8	54	42	57	51	52	46	53
day 15	49	43	58	54	59	57	73
day 22	41	37	57	47	59	55	64
day 29	44	36	53	43	44	45	42
day 36	37	39	57	47	58	61	72
day 43	40	30	50	45	57	57	61
day 50	36	31	45	46	49	61	62
day 57	41	32	49	44	57	54	n.d.
day 64	40	30	41	37	49	55	n.d.
day 71	38	28	27	26	42	34	n.d.
day 78	42	35	42	39	48	51	n.d.
day 85	30	22	60	40	39	36	n.d.

n.d.=no data

Table 140
Effect of antisense oligonucleotide treatment on lymphocytes (%) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	54	59	47	58	42	53	47
day -5	56	59	43	49	44	53	43
day 8	43	54	39	45	45	50	44
day 15	47	53	38	43	38	40	24
day 22	54	59	39	49	37	41	33
day 29	51	59	43	51	51	50	53
day 36	58	57	39	49	38	35	26
day 43	55	65	45	51	39	39	36
day 50	59	64	49	48	46	34	35
day 57	55	63	45	51	39	40	n.d.
day 64	56	64	53	56	46	39	n.d.
day 71	56	65	61	66	52	59	n.d.
day 78	53	60	51	54	46	41	n.d.
day 85	63	72	34	52	54	56	n.d.

n.d.=no data

Table 141
Effect of antisense oligonucleotide treatment on eosinophils (%) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	1.3	0.6	1.0	0.7	1.0	0.3	0.5
day -5	1.5	0.6	1.6	1.3	0.9	0.3	0.7
day 8	0.9	0.4	1.1	0.3	0.7	0.2	0.5
day 15	0.7	0.3	1.0	0.3	0.5	0.1	0.2
day 22	0.9	0.5	0.7	0.6	0.9	0.3	0.5
day 29	0.9	0.3	1.2	0.6	0.9	0.3	0.8
day 36	0.9	0.5	1.7	0.4	0.6	0.2	0.4
day 43	0.9	0.6	1.2	0.3	0.6	0.2	0.4
day 50	1.2	0.8	1.2	0.4	0.7	0.1	0.3
day 57	0.7	0.6	1.0	0.3	0.4	0.2	n.d.
day 64	1.0	0.7	1.3	0.4	0.7	0.2	n.d.
day 71	1.6	0.8	1.8	0.9	1.1	0.3	n.d.
day 78	1.0	0.9	1.0	0.5	1.2	0.1	n.d.
day 85	1.3	1.5	1.2	0.6	1.6	0.2	n.d.

n.d.=no data

Table 142
Effect of antisense oligonucleotide treatment on monocytes (%) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	3.3	3.1	2.3	2.8	2.8	3.0	2.9
day -5	3.8	3.6	2.8	2.8	3.3	3.2	2.4
day 8	2.3	2.5	1.8	2.7	2.1	3.3	1.8
day 15	2.7	2.4	2.0	2.2	2.4	2.3	1.5
day 22	3.4	2.9	2.4	2.8	2.8	3.1	1.9
day 29	3.3	3.2	2.7	3.8	3.4	3.5	2.7
day 36	3.1	2.5	2.1	2.9	2.3	2.6	1.5
day 43	3.5	3.3	2.6	3.1	2.1	2.8	1.8
day 50	2.6	3.2	3.7	4.6	2.9	3.1	1.8
day 57	2.6	3.2	n.d.3.2	3.8	2.4	3.6	n.d.
day 64	2.6	3.5	n.d.3.5	4.4	2.8	4.0	n.d.
day 71	3.4	4.3	n.d.4.7	4.9	3.7	4.7	n.d.
day 78	3.3	3.6	n.d.4.5	4.9	3.7	4.7	n.d.
day 85	4.4	3.7	n.d.3.5	6.1	3.7	5.3	n.d.

n.d.=no data

Table 143

Effect of antisense oligonucleotide treatment on basophils (%) in cynomolgus monkeys

	PBS	ISIS 416838	ISIS 416850	ISIS 416858	ISIS 416864	ISIS 417002	ISIS 416850*
day -14	0.3	0.2	0.2	0.3	0.2	0.3	0.2
day -5	0.3	0.3	0.2	0.3	0.2	0.3	0.3
day 8	0.2	0.2	0.2	0.3	0.2	0.3	0.3
day 15	0.3	0.3	0.2	0.2	0.2	0.2	0.2
day 22	0.2	0.2	0.2	0.2	0.2	0.2	0.1
day 29	0.3	0.2	0.2	0.2	0.3	0.2	0.3
day 36	0.3	0.4	0.3	0.3	0.3	0.2	0.1
day 43	0.3	0.4	0.3	0.3	0.4	0.3	0.2
day 50	0.4	0.3	0.3	0.4	0.4	0.3	0.2
day 57	0.2	0.3	0.4	0.2	0.3	0.3	n.d.
day 64	0.3	0.4	0.4	0.4	0.4	0.2	n.d.
day 71	0.2	0.5	0.3	0.4	0.4	0.3	n.d.
day 78	0.2	0.4	0.3	0.4	0.3	0.3	n.d.
day 85	0.3	0.3	0.3	0.3	0.4	0.3	n.d.

n.d.=no data

Cytokine and chemokine assays

Blood samples obtained from the monkey groups treated with PBS, ISIS 416850 and ISIS 416858 administered in the escalating dose regimen were sent to Pierce Biotechnology (Woburn, MA) for measurement of chemokine and cytokine levels. Levels of IL-1β, IL-6, IFN-γ, and TNF-α were measured using the respective primate antibodies and levels of IL-8, MIP-1α, MCP-1, MIP-1β and RANTES were measured using the respective cross-reacting human antibodies. Measurements were taken 14 days before the start of treatment and on day 85, when the monkeys were euthanized. The results are presented in Tables 144 and 145.

Table 144

Effect of antisense oligonucleotide treatment on cytokine/chemokine levels (pg/mL) in cynomolgus monkeys on day -14

	IL-1β	IL-6	IFN-γ	TNF-α	IL-8	MIP-1α	MCP-1	MIP-1β	RANTES
PBS	16	10	114	7	816	54	1015	118	72423
ISIS 416850	3	30	126	14	1659	28	1384	137	75335
ISIS 416858	5	9	60	9	1552	36	1252	122	112253

Table 145

Effect of antisense oligonucleotide treatment on cytokine/chemokine levels (pg/mL) in cynomolgus monkeys on day 85

	IL-1β	IL-6	IFN-γ	TNF-α	IL-8	MIP-1α	MCP-1	MIP-1β	RANTES
PBS	7	4	102	34	87	23	442	74	84430
ISIS 416850	13	17	18	27	172	41	2330	216	83981
ISIS 416858	5	25	18	45	303	41	1752	221	125511

Example 46: Pharmacologic effect of antisense oligonucleotides targeting human Factor 11 in cynomolgus monkeys

Several antisense oligonucleotides chosen from the rodent tolerability studies (Examples 41-44) were tested in cynomolgus monkeys to determine their pharmacologic effects, relative efficacy on Factor 11 activity and tolerability in a cynomolgus monkey model. The antisense oligonucleotides were also compared to ISIS 416850 and ISIS 416858 selected from the monkey study described earlier (Example 45). All the ISIS oligonucleotides used in this study target human Factor 11 mRNA and are also fully cross-reactive with the rhesus monkey gene sequence (see Tables 44 and 46). It is expected that the rhesus monkey ISIS oligonucleotides are fully cross-reactive with the cynomolgus monkey gene sequence as well. At the time the study was undertaken, the cynomolgus monkey genomic sequence was not available in the National Center for Biotechnology Information (NCBI) database; therefore, cross-reactivity with the cynomolgus monkey gene sequence could not be confirmed.

Treatment

Groups, each consisting of two male and two female monkeys, were injected subcutaneously with 25 mg/kg of ISIS 416850, ISIS 449709, ISIS 445522, ISIS 449710, ISIS 449707, ISIS 449711, ISIS 449708, 416858, and ISIS 445531. Antisense oligonucleotide was administered to the monkeys at 25 mg/kg three times per week for week 1 and 25 mg/kg twice per week for weeks 2 to 8. A control group, consisting of two male and two female monkeys was injected subcutaneously with PBS according to the same dosing regimen. Body weights were taken 14 days and 7 days before the start of treatment and were then measured weekly throughout the treatment period. Blood samples were collected 14 days and 5 days before the start of treatment and subsequently several times during the dosing regimen for PT and aPTT measurements, and measurement of various hematologic factors. On day 55, the monkeys were euthanized by exsanguination while under deep anesthesia, and organs harvested for further analysis.

RNA analysis

On day 55, RNA was extracted from liver tissue for real-time PCR analysis of Factor 11 using primer probe set LTS00301. Results are presented as percent inhibition of Factor 11, relative to PBS control. As shown in Table 146, treatment with ISIS 416850, ISIS 449709, ISIS 445522, ISIS 449710, ISIS 449707, ISIS 449708, ISIS 416858, and ISIS 445531 resulted in significant reduction of Factor 11 mRNA in comparison to the PBS control.

Table 146

Inhibition of Factor 11 mRNA in the cynomolgus monkey liver relative to the PBS control

Oligo ID	% inhibition
416850	68
449709	69
445522	89
449710	52
449707	47
449711	0
449708	46
416858	89
445531	66

Protein analysis

Plasma samples from all monkey groups taken on different days were analyzed by a sandwich-style ELISA assay (Affinity Biologicals Inc.) using an affinity-purified polyclonal anti-Factor 11 antibody as the capture antibody and a peroxidase-conjugated polyclonal anti-Factor 11 antibody as the detecting antibody. Monkey plasma was diluted 1:50 for the assay. Peroxidase activity was expressed by incubation with the substrate o-phenylenediamine. The color produced was quantified using a microplate reader at 490 nm and was considered to be proportional to the concentration of Factor 11 in the samples.

The results are presented in Table 147, expressed as percentage reduction relative to that of the PBS control. Treatment with ISIS 416850, ISIS 449709, ISIS 445522, and ISIS 416858 resulted in a time-dependent decrease in protein levels.

Table 147
Inhibition of Factor 11 protein in the cynomolgus monkey liver relative to the PBS control

ISIS No.	Day -14	Day -5	Day 10	Day 17	Day 24	Day 31	Day 38	Day 45	Day 52	Day 55
416850	0	0	20	31	38	52	51	53	53	58
449709	1	0	27	35	44	45	46	48	47	50
445522	2	0	36	50	61	70	73	77	80	82
449710	1	0	10	14	17	25	20	23	4	24
449707	0	0	16	19	21	29	28	35	29	32
449711	0	1	5	3	6	9	2	4	3	5
449708	1	0	7	15	3	14	9	2	6	6
416858	4	0	36	49	62	68	74	79	81	81
445531	0	1	9	22	23	27	29	32	32	37

PT and aPTT assay

PT and aPTT were measured using platelet poor plasma (PPP) from mice treated with ISIS oligonucleotides. PT and aPTT values are provided in Tables 148 and 149 and are reported as International Normalized Ratio (INR) values. INR values for PT and aPTT were determined by dividing the PT or aPTT value for each experimental group by the PT or aPTT for the PBS treated group. This ratio was then raised to the power of the International Sensitivity Index (ISI) of the tissue factor used. As shown in Table 148, PT was not significantly prolonged in mice treated with ISIS oligonucleotides. However, aPTT was significantly prolonged in groups treated with ISIS 416850, ISIS 445522, and ISIS 416858, as presented in Table 149. These data suggest that antisense reduction of Factor 11 affects the contact activation pathway, but not the extrinsic pathway of blood coagulation. Therefore, antisense reduction of Factor 11 with these ISIS oligonucleotides is useful for inhibiting the formation of a thrombus or clot in response to an abnormal vessel wall, but not in response to tissue injury.

Table 148
Effect of antisense oligonucleotide treatment on PT ratio in cynomolgus monkeys

	Day -14	Day -5	Day 10	Day 17	Day 24	Day 31	Day 38	Day4 5	Day 52	Day 55
ISIS 416850	1.02	1.00	0.99	1.00	0.97	1.00	1.01	1.00	1.02	1.07
ISIS 449709	1.00	0.96	0.95	0.95	0.95	0.95	0.97	0.97	0.99	1.03
ISIS 445522	1.00	0.94	0.95	0.96	0.94	0.96	0.97	0.96	0.98	1.01
ISIS 449710	1.03	0.96	0.98	1.00	0.97	0.98	0.99	0.97	0.98	1.06
ISIS 449707	1.01	0.94	0.95	0.97	0.95	0.96	1.00	0.96	0.96	1.00

ISIS 449711	1.00	0.95	0.94	0.95	0.94	0.98	1.02	1.01	1.00	1.07
ISIS 449708	1.03	0.95	0.98	1.00	0.95	1.06	0.99	0.99	0.99	1.04
ISIS 416858	1.01	0.96	0.96	0.98	0.95	1.00	0.97	1.00	0.99	1.01
ISIS 445531	1.06	1.00	1.00	1.06	1.02	1.04	1.03	1.01	1.04	1.06

Table 149

Effect of antisense oligonucleotide treatment on aPTT ratio in cynomolgus monkeys

	Day -14	Day -5	Day 10	Day 17	Day 24	Day 31	Day 38	Day 45	Day 52	Day 55
ISIS 416850	0.99	0.90	0.98	1.01	1.05	1.22	1.25	1.34	1.32	1.45
ISIS 449709	0.99	0.91	0.99	1.03	1.05	1.08	1.08	1.15	1.09	1.17
ISIS 445522	0.96	0.91	1.06	1.10	1.14	1.25	1.32	1.39	1.39	1.42
ISIS 449710	1.07	0.98	1.00	0.97	1.00	1.04	1.02	1.06	1.03	1.07
ISIS 449707	0.90	0.87	0.92	0.94	0.93	0.95	0.99	1.00	0.99	1.04
ISIS 449711	0.94	0.96	0.92	0.90	0.92	0.89	0.93	0.94	0.92	0.96
ISIS 449708	1.07	1.01	1.06	1.05	1.01	1.09	1.06	1.06	1.08	1.11
ISIS 416858	1.03	0.96	1.07	1.13	1.21	1.32	1.41	1.49	1.53	1.61
ISIS 445531	1.00	0.89	0.95	1.05	1.00	1.07	1.06	1.13	1.15	1.19

Body and organ weights

Body weights of each group are given in Table 150 expressed in grams. The results indicate that treatment with the antisense oligonucleotides did not cause any adverse changes in the health of the animals, which may have resulted in a significant alteration in weight compared to the PBS control. Organ weights were taken after the animals were euthanized on day 55, and livers, kidneys and spleens were harvested. The results are presented in Table 150 expressed as a percentage of the body weight and also show no significant alteration in weights compared to the PBS control, with the exception of ISIS 449711, which caused increase in spleen weight.

Table 150

Weekly measurements of body weights (g) of cynomolgus monkeys

Days	PBS	ISIS 416850	ISIS 449709	ISIS 445522	ISIS 449710	ISIS 449707	ISIS 449711	ISIS 449708	ISIS 416858	ISIS 445531
-14	2069	2061	2044	2050	2097	2072	2049	2096	2073	2079
-7	2107	2074	2093	2042	2114	2083	2105	2163	2092	2092
1	2131	2083	2112	2047	2131	2107	2123	2130	2115	2125
8	2186	2072	2075	2094	2120	2088	2123	2148	2149	2119
15	2201	2147	2085	2092	2145	2120	2103	2125	2162	2109

22	2206	2139	2117	2114	2177	2142	2171	2110	2188	2143
29	2204	2159	2068	2125	2149	2155	2203	2095	2196	2148
36	2246	2136	2064	2121	2180	2158	2227	2100	2210	2191
43	2304	2186	2106	2142	2227	2197	2251	2125	2238	2233
50	2274	2143	2147	2127	2201	2185	2227	2076	2225	2197

Table 151
Organ weights (g) of cynomolgus monkeys after antisense oligonucleotide treatment

	Liver	Spleen	Kidney
PBS	2.3	0.16	0.48
ISIS 416850	2.5	0.17	0.51
ISIS 449709	2.6	0.21	0.57
ISIS 445522	2.6	0.23	0.55
ISIS 449710	2.6	0.24	0.58
ISIS 449707	2.5	0.24	0.53
ISIS 449711	2.6	0.32	0.54
ISIS 449708	2.6	0.19	0.60
ISIS 416858	2.6	0.24	0.47
ISIS 445531	2.8	0.24	0.49

Liver function

To evaluate the impact of ISIS oligonucleotides on hepatic function, plasma concentrations of ALT and AST were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of alanine transaminase (ALT) and aspartate transaminase (AST) were measured and the results are presented in Tables 152 and 153 expressed in IU/L. Plasma levels of bilirubin were also measured and results are presented in Table 154 expressed in mg/dL. As observed in Tables 152-154, there were no significant increases in any of the liver metabolic markers after antisense oligonucleotide treatment.

Table 152
Effect of antisense oligonucleotide treatment on ALT (IU/L) in the liver of cynomolgus monkeys

	Day -14	Day -5	Day 31	Day 55
PBS	57	55	53	57
ISIS 416850	48	42	45	55
ISIS 449709	73	77	65	102
ISIS 445522	43	45	40	60
ISIS 449710	37	42	37	45

ISIS 449707	54	56	52	63
ISIS 449711	49	137	48	54
ISIS 449708	48	54	44	46
ISIS 416858	43	66	46	58
ISIS 445531	84	73	57	73

Table 153

Effect of antisense oligonucleotide treatment on AST (IU/L) in the liver of cynomolgus monkeys

	Day -14	Day -5	Day 31	Day 55
PBS	65	45	44	47
ISIS 416850	62	45	46	57
ISIS 449709	62	51	45	71
ISIS 445522	62	47	46	79
ISIS 449710	52	38	37	64
ISIS 449707	64	53	50	52
ISIS 449711	58	78	47	47
ISIS 449708	74	53	56	50
ISIS 416858	64	100	60	69
ISIS 445531	78	46	47	49

Table 154

Effect of antisense oligonucleotide treatment on bilirubin (mg/dL) in the liver of cynomolgus monkeys

	Day -14	Day -5	Day 31	Day 55
PBS	0.25	0.20	0.20	0.17
ISIS 416850	0.26	0.22	0.26	0.17
ISIS 449709	0.24	0.19	0.15	0.18
ISIS 445522	0.24	0.20	0.14	0.18
ISIS 449710	0.24	0.19	0.15	0.22
ISIS 449707	0.27	0.19	0.13	0.16
ISIS 449711	0.23	0.16	0.13	0.13
ISIS 449708	0.27	0.21	0.14	0.14
ISIS 416858	0.25	0.23	0.16	0.16
ISIS 445531	0.22	0.18	0.13	0.11

Kidney function

To evaluate the impact of ISIS oligonucleotides on kidney function, urine samples were collected on different days. BUN levels were measured at various time points using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY) and the results are presented in Table 155. The ratio of urine protein to creatinine in urine samples after antisense oligonucleotide treatment was also calculated for day 49 and results are presented in Table 156. As observed in Tables 155 and 156, there were no significant increases in any of the kidney metabolic markers after antisense oligonucleotide treatment.

Table 155

Effect of antisense oligonucleotide treatment on BUN levels (mg/dL) in cynomolgus monkeys

	Day -14	Day -5	Day 31	Day 55
PBS	22	21	22	22
ISIS 416850	24	23	21	26
ISIS 449709	22	21	20	28
ISIS 445522	23	22	22	22
ISIS 449710	19	19	19	23
ISIS 449707	25	21	21	20
ISIS 449711	26	22	20	23
ISIS 449708	25	23	23	23
ISIS 416858	25	24	23	24
ISIS 445531	22	18	20	22

Table 156

Effect of antisense oligonucleotide treatment on urine protein to creatinine ratio in cynomolgus monkeys

	Urine protein/creatinine ratio
PBS	0.02
ISIS 416850	0.08
ISIS 449709	0.05
ISIS 445522	0.01
ISIS 449710	0.00
ISIS 449707	0.03
ISIS 449711	0.01
ISIS 449708	0.00
ISIS 416858	0.05
ISIS 445531	0.08

Hematology assays

Blood obtained from all the monkey groups on different days were sent to Korea Institute of Toxicology (KIT) for HCT, MCV, MCH, and MCHC measurements, as well as measurements of the various blood cells, such as WBC (neutrophils and monocytes), RBC and platelets, as well as total hemoglobin content. The results are presented in Tables 157-166.

Table 157

Effect of antisense oligonucleotide treatment on HCT (%) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	40	42	43	43	41	40
ISIS 416850	41	44	42	42	42	40
ISIS 449709	41	42	43	42	41	40
ISIS 445522	42	42	41	43	41	39
ISIS 449710	41	44	43	44	43	41
ISIS 449707	40	43	42	43	43	42
ISIS 449711	41	41	42	39	39	38
ISIS 449708	41	44	44	43	44	42
ISIS 416858	41	44	43	43	41	39
ISIS 445531	41	42	43	41	41	41

Table 158

Effect of antisense oligonucleotide treatment on platelet count (x 100/µL) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	361	441	352	329	356	408
ISIS 416850	462	517	467	507	453	396
ISIS 449709	456	481	449	471	418	441
ISIS 445522	433	512	521	425	403	333
ISIS 449710	411	463	382	422	313	360
ISIS 449707	383	464	408	408	424	399
ISIS 449711	410	431	325	309	257	259
ISIS 449708	387	517	444	378	381	348
ISIS 416858	369	433	358	289	287	257
ISIS 445531	379	416	380	376	345	319

Table 159

Effect of antisense oligonucleotide treatment on neutrophils (%) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	81	84	75	75	91	118
ISIS 416850	88	109	95	100	85	108
ISIS 449709	73	101	89	81	77	115
ISIS 445522	61	84	81	66	69	125
ISIS 449710	93	86	80	94	97	132
ISIS 449707	85	106	80	89	89	98

ISIS 449711	64	71	52	58	45	70
ISIS 449708	73	84	61	57	61	75
ISIS 416858	65	84	54	54	61	73
ISIS 445531	60	80	85	116	93	91

Table 160

Effect of antisense oligonucleotide treatment on monocytes (%) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	1.9	2.8	3.1	2.8	3.9	2.2
ISIS 416850	1.9	2.9	3.2	3.7	3.8	3.4
ISIS 449709	4.0	2.0	3.0	2.8	3.6	3.4
ISIS 445522	2.1	2.3	3.6	3.9	4.4	3.0
ISIS 449710	1.3	2.0	2.5	2.4	3.4	1.6
ISIS 449707	1.3	2.3	3.2	4.2	4.0	4.8
ISIS 449711	1.2	2.3	5.9	6.9	7.6	7.8
ISIS 449708	1.7	2.6	5.4	5.8	7.0	6.2
ISIS 416858	2.0	2.7	4.0	4.7	4.6	4.6
ISIS 445531	1.3	2.2	3.4	4.1	4.4	4.1

Table 161

Effect of antisense oligonucleotide treatment on hemoglobin content (g/dL) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	12.3	12.5	12.9	12.7	12.4	12.1
ISIS 416850	13.0	13.5	13.3	13.1	13.1	12.7
ISIS 449709	12.8	12.8	13.2	13.1	12.6	12.5
ISIS 445522	13.3	12.7	12.7	12.9	12.6	12.0
ISIS 449710	13.0	13.2	13.4	13.1	13.0	12.7
ISIS 449707	12.7	12.8	12.7	12.7	12.9	12.6
ISIS 449711	12.7	12.7	12.5	11.8	11.5	11.3
ISIS 449708	13.0	13.2	13.5	13.0	13.3	13.0
ISIS 416858	12.8	13.0	13.0	12.8	12.3	12.0
ISIS 445531	12.6	12.6	12.7	12.3	12.0	12.1

Table 162

Effect of antisense oligonucleotide treatment on WBC count ($x10^3/\mu L$) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	10	10	11	12	11	12
ISIS 416850	12	13	11	12	12	10
ISIS 449709	11	10	11	11	11	10
ISIS 445522	10	9	11	13	10	11
ISIS 449710	11	11	12	12	11	15

ISIS 449707	13	11	12	11	12	8
ISIS 449711	13	12	10	9	9	7
ISIS 449708	14	10	11	11	10	10
ISIS 416858	10	11	10	9	8	9
ISIS 445531	20	15	17	17	20	15

Table 163

Effect of antisense oligonucleotide treatment on RBC count (x10^6/μL) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	5.6	5.6	5.8	5.8	5.6	5.5
ISIS 416850	5.5	5.7	5.6	5.6	5.7	5.6
ISIS 449709	5.8	5.8	5.9	5.9	5.7	5.7
ISIS 445522	5.9	5.6	5.6	5.8	5.7	5.4
ISIS 449710	5.6	5.8	5.8	5.8	5.7	5.6
ISIS 449707	5.7	5.8	5.7	5.7	5.9	5.8
ISIS 449711	5.6	5.7	5.6	5.4	5.4	5.3
ISIS 449708	5.7	5.9	5.9	5.8	6.0	5.8
ISIS 416858	5.5	5.5	5.6	5.6	5.5	5.3
ISIS 445531	5.7	5.7	5.8	5.6	5.5	5.6

Table 164

Effect of antisense oligonucleotide treatment on MCV (fL) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	72	74	75	73	73	73
ISIS 416850	74	77	76	75	75	73
ISIS 449709	72	74	73	73	71	71
ISIS 445522	72	74	74	75	73	72
ISIS 449710	75	77	75	75	75	73
ISIS 449707	71	75	74	74	73	73
ISIS 449711	73	74	75	73	73	73
ISIS 449708	73	75	75	75	74	74
ISIS 416858	75	79	78	76	75	75
ISIS 445531	72	74	75	75	75	74

Table 165

Effect of antisense oligonucleotide treatment on MCH (pg) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	22.1	22.4	22.3	22.1	22.0	22.0
ISIS 416850	23.7	23.7	23.7	23.3	22.7	22.9
ISIS 449709	22.4	22.3	22.5	22.2	21.0	22.0
ISIS 445522	22.6	22.5	22.8	22.4	22.4	22.2

ISIS 449710	23.0	22.8	23.1	22.6	21.8	22.7
ISIS 449707	22.2	22.2	22.1	22.1	22.6	21.9
ISIS 449711	22.6	22.7	22.2	22.1	21.7	21.3
ISIS 449708	22.9	22.7	22.9	22.7	22.2	22.5
ISIS 416858	23.2	23.5	23.1	23.0	22.2	22.8
ISIS 445531	22.2	22.2	22.1	22.0	21.6	21.7

Table 166

Effect of antisense oligonucleotide treatment on MCHC (g/dL) in cynomolgus monkeys

	Day -14	Day -5	Day 17	Day 31	Day 45	Day 55
PBS	30.8	30.0	30.1	29.9	30.3	30.2
ISIS 416850	32.0	30.7	31.3	31.0	31.0	30.9
ISIS 449709	31.4	30.3	30.7	30.7	31.1	31.2
ISIS 445522	31.4	30.4	30.9	30.0	30.7	31.0
ISIS 449710	31.2	29.7	30.7	30.1	30.4	31.1
ISIS 449707	31.4	29.8	30.0	29.8	29.8	30.0
ISIS 449711	31.0	30.7	29.9	29.8	29.6	29.5
ISIS 449708	31.4	30.2	30.7	29.9	30.6	31.8
ISIS 416858	31.1	29.8	29.9	31.0	30.3	30.4
ISIS 445531	30.9	30.0	29.5	29.7	29.0	29.6

Cytokine and chemokine assays

Blood samples obtained from all monkey groups were sent to Pierce Biotechnology (Woburn, MA) for measurements of chemokine and cytokine levels. Levels of IL-1β, IL-6, IFN-γ, and TNF-α were measured using the respective primate antibodies and levels of IL-8, MIP-1α, MCP-1, MIP-1β and RANTES were measured using the respective cross-reacting human antibodies. Measurements were taken 14 days before the start of treatment and on day 55, when the monkeys were euthanized. The results are presented in Tables 167 and 168.

Table 167

Effect of antisense oligonucleotide treatment on cytokine/chemokine levels (pg/mL) in cynomolgus monkeys on day -14

	IL-1β	IL-6	IFN-γ	TNF-α	IL-8	MIP-1α	MCP-1	MIP-1β	RANTES
PBS	350	3	314	32	82	27	277	8	297
ISIS 416850	215	1	115	4	45	14	434	31	4560
ISIS 449409	137	1	37	9	34	13	290	14	2471
ISIS 445522	188	5	172	16	32	22	297	27	3477
ISIS 449710	271	7	1115	72	29	20	409	18	1215
ISIS 449707	115	1	34	6	106	16	294	13	3014

ISIS 449711	79	2	29	6	156	20	264	24	3687
ISIS 449708	35	1	27	12	184	11	361	19	11666
ISIS 416858	103	0	32	4	224	11	328	37	6521
ISIS 445531	101	2	68	9	83	25	317	22	7825

Table 168

Effect of antisense oligonucleotide treatment on cytokine/chemokine levels (pg/mL) in cynomolgus monkeys on day 55

	IL-1β	IL-6	IFN-γ	TNF-α	IL-8	MIP-1α	MCP-1	MIP-1β	RANTES
PBS	453	3	232	191	68	21	237	34	775
ISIS 416850	106	1	19	16	620	17	887	50	27503
ISIS 449409	181	0	25	8	254	17	507	47	8958
ISIS 445522	341	2	83	18	100	22	592	63	16154
ISIS 449710	286	2	176	26	348	27	474	53	22656
ISIS 449707	97	1	24	16	48	12	264	49	1193
ISIS 449711	146	7	22	31	110	17	469	91	3029
ISIS 449708	131	0	18	17	85	23	409	128	4561
ISIS 416858	28	1	9	15	167	11	512	47	5925
ISIS 445531	155	1	15	16	293	12	339	84	5935

Example 47: Measurement of viscosity of ISIS antisense oligonucleotides targeting human Factor 11

The viscosity of antisense oligonucleotides targeting human Factor 11 was measured with the aim of screening out antisense oligonucleotides which have a viscosity more than 40 cP at a concentration of 165-185 mg/mL.

ISIS oligonucleotides (32-35 mg) were weighed into a glass vial, 120 µL of water was added and the antisense oligonucleotide was dissolved into solution by heating the vial at 50^0C. Part of (75 µL) the pre-heated sample was pipetted to a micro-viscometer (Cambridge). The temperature of the micro-viscometter was set to 25^0C and the viscosity of the sample was measured. Another part

(20 μL) of the pre-heated sample was pipetted into 10 mL of water for UV reading at 260 nM at 85^0C (Cary UV instrument). The results are presented in Table 169.

Table 169

Viscosity and concentration of ISIS antisense oligonucleotides targeting human Factor 11

ISIS No.	Viscosity (cP)	Concentration (mg/mL)
412223	8	163
412224	98	186
412225	> 100	162
413481	23	144
413482	16.	172
416848	6	158
416850	67	152
416851	26	187
416852	29	169
416856	18	175
416858	10	166
416859	10	161
416860	> 100	154
416861	14	110
416863	9	165
416866	> 100	166
416867	8	168
445498	21	157
445504	20	139
445505	9	155
445509	> 100	167
445513	34	167
445522	63	173
445522	58	174
445530	25	177
445531	15	155
445531	20	179
449707	7	166
449708	9	188
449709	65	171
449710	7	186
449711	6	209
451541	10	168

CLAIMS

What is claimed is:

1. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 223.

2. The compound of claim 1, consisting of a single-stranded modified oligonucleotide.

3. The compound of claim 2, wherein the nucleobase sequence of the modified oligonucleotide is 100% complementary to a nucleobase sequence of SEQ ID NO: 1.

4. The compound of claim 2, wherein at least one internucleoside linkage is a modified internucleoside linkage.

5. The compound of claim 4, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

6. The compound of claim 1, wherein at least one nucleoside comprises a modified sugar.

7. The compound of claim 6, wherein at least one modified sugar is a bicyclic sugar.

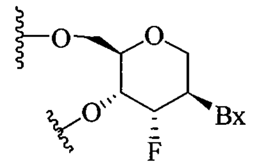
8. The compound of claim 7, wherein each of the at least one bicyclic sugar comprises a 4'-$(CH_2)_n$-O-2' bridge, wherein n is 1 or 2.

9. The compound of claim 7, wherein each of the at least one bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

10. The compound of claim 6, wherein at least one modified sugar comprises a 2'-O-methoxyethyl group.

11. The compound of claim 1, comprising at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

12. The compound of claim 11, wherein each of the at least one tetrahydropyran modified nucleoside has the structure:



wherein Bx is an optionally protected heterocyclic base moiety.

13. The compound of claim 2, wherein at least one nucleoside comprises a modified nucleobase.

14. The compound of claim 13, wherein the modified nucleobase is a 5-methylcytosine.

15. The compound of claim 1, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides;

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

16. The compound of claim 15, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides;

a 3' wing segment consisting of five linked nucleosides;

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

17. The compound of claim 16, wherein each cytosine is a 5-methylcytosine.

18. The compound of claim 2 or 17, wherein the modified oligonucleotide consists of 20 linked nucleosides.

19. A compound according to any one of claims 1 to 18 for treating thromboembolic complications.

20. The compound of claim 19, wherein the animal is a human.

21. The compound of claim 20, for preventing deep vein thrombosis.

22. The compound of any one of claims 20, for preventing pulmonary embolism.

23. The compound of any one of claims 19, for coadministration with any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, LOVENOX, and Factor Xa inhibitor.

24. The compound of any one of claims 19, for concomitant administration with any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, LOVENOX, and Factor Xa inhibitor.

25. The compound of claim 23, for parental administration.

26. The compound of claim 24, for parental administration

27. The compound of claim 25, for subcutaneous or intravenous administration.

28. The compound of claim 26, for subcutaneous or intravenous administration.

29. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified oligonucleotide is complementary to a Factor 11 nucleic acid for treating a clotting disorder.

30. Use of the compound of any one of claims 1 to 18 with any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, and LOVENOX for the manufacture of a medicament for treating thromboembolic complications.

31. A composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 223 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

32. The composition of claim 31, consisting of a single-stranded oligonucleotide.

33. The composition of claim 32, wherein the modified oligonucleotide consists of 20 linked nucleosides.

34. A method comprising identifying an animal at risk for thromboembolic complications; and

administering to the at risk 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 11 nucleic acid.

35. The method of claim 34, wherein the thromboembolic complication is deep vein thrombosis, pulmonary embolism, or a combination thereof.

36. A method comprising identifying an animal having a clotting disorder; and

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 11 nucleic acid.

37. The method of claim 36, comprising co-administering the compound and any of the group selected from aspirin, clopidogrel, dipyridamole, heparin, lepirudin, ticlopidine, warfarin, apixaban, rivaroxaban, LOVENOX, and Factor Xa inhibitor.

38. A method comprising reducing the risk for thromboembolic complications in an animal; and

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 11 nucleic acid.

39. A method comprising treating a clotting disorder in an animal; and

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 11 nucleic acid.

40. The method of claim 39, comprising reversing Factor 11 inhibition in the animal by administering an antidote to the modified oligonucleotide.

41. The method of claim 39, wherein the antidote is an oligonucleotide complementary to the modified oligonucleotide.

42. The method of claim 40, comprising reversing Factor 11 inhibition in the animal by administering any of the group selected from Factor 7, Factor 7a, Factor 11, or Factor 11a protein.

43. A method comprising identifying an animal at risk for thromboembolic complications; and

administering to the at risk animal a therapeutically effective amount of a Factor 11 specific inhibitor and anti-platelet therapy.

44. A method comprising identifying an animal having a clotting disorder; and

administering to the animal a therapeutically effective amount of a Factor 11 specific inhibitor and anti-platelet therapy.

45. A method comprising reducing the risk for thromboembolic complications in an animal; and

administering to the animal a therapeutically effective amount of a Factor 11 specific inhibitor and anti-platelet therapy.

46. A method comprising treating a clotting disorder in an animal; and

administering to the animal a therapeutically effective amount of a Factor 11 specific inhibitor and anti-platelet therapy.

47. The method of any of claims 43-46, wherein the anti-platelet therapy is selected from an ADP receptor inhibitor, NSAID, phosphodiesterase inhibitor, glycoprotein IIB/IIIA inhibitor or adenosine reuptake inhibitor or a combination therof.

48. The method of claim 47, wherein the NSAID is aspirin, naproxen or a combination of both.

49. The method of claim 47, wherein the ADPreceptor/P2Y12 inhibitor is a Thienopyridine

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50. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 738 to 762 of SEQ ID NO: 1; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

51. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 656 to 704 of SEQ ID NO: 1; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

52. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1018 to 1042 of SEQ ID NO: 1; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

53. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1062 to 1091 of SEQ ID NO: 1; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

54. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1275 to 1318 of SEQ ID NO: 1; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.