



(43) International Publication Date
15 August 2013 (15.08.2013)

(10) International Publication Number
WO 2013/119979 A1

- (51) **International Patent Classification:**
C12N 15/11 (2006.01) *C07H 21/04* (2006.01)
- (21) **International Application Number:**
PCT/US2013/025381
- (22) **International Filing Date:**
8 February 2013 (08.02.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/596,688 8 February 2012 (08.02.2012) US
- (71) **Applicant:** ISIS PHARMACEUTICALS, INC. [US/US];
2855 Gazelle Court, Carlsbad, CA 92010 (US).
- (72) **Inventors:** SWAYZE, Eric, E.; 2855 Gazelle Court,
Carlsbad, CA 92010 (US). FREIER, Susan, M.; 2855
Gazelle Court, Carlsbad, CA 92010 (US).
- (74) **Agents:** GINGRICH, Brenden et al.; Knobbe Martens,
2040 Main Street, 14th Floor, Irvine, CA 92614 (US).
- (81) **Designated States** (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,

DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,
RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.

- (84) **Designated States** (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) **Title:** METHODS AND COMPOSITIONS FOR MODULATING FACTOR VII EXPRESSION

(57) **Abstract:** Disclosed herein are antisense compounds and methods for decreasing Factor VII and treating, preventing, or slowing progression of thromboembolic complications, hyperproliferative disorders, or inflammatory conditions in an individual in need thereof.



WO 2013/119979 A1

METHODS AND COMPOSITIONS FOR MODULATING FACTOR VII EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0169WOSEQ.txt created February 6, 2013, which is 164 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

Embodiments described herein provide methods, compounds, and compositions for reducing expression of Factor VII mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate thromboembolic complications, hyperproliferative disorders, and inflammatory conditions.

Background

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 unfractionated 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

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

In certain embodiments, modulation occurs 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 VII mRNA levels are reduced. In certain embodiments, Factor VII protein levels are reduced. In certain embodiments, both Factor VII mRNA and protein levels are reduced. Such reduction may occur in a time-dependent or in a dose-dependent manner.

Also provided are methods for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such diseases, disorders, and conditions are thromboembolic complications, hyperproliferative disorders, and inflammatory conditions. Certain such thromboembolic complications include thrombosis, embolism, and thromboembolism, such as, deep vein thrombosis, pulmonary embolism, myocardial infarction, stroke, cancer, rheumatoid arthritis, and fibrosis. Certain such hyperproliferative disorders include cancer, psoriasis, hyperplasia and the like. Certain such inflammatory conditions include rheumatoid arthritis, liver fibrosis, sepsis, myocardial ischemia/reperfusion injury, adult respiratory distress syndrome, nephritis, graft rejection, inflammatory bowel disease, multiple sclerosis, arteriosclerosis, and vasculitis.

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 of the individual. Certain risk factors and causes for development of a hyperproliferative disorder include genetic factors, such as gene mutations and chromosomal aberrations, which may or may not be inherited; and environmental factors, which include, but are not limited to, exposure to known mutagens, such as high energy radiation from radioactive elements, X-rays, gamma rays, microwaves, and ultraviolet

light; certain industrial chemicals; pollutants such as cigarette smoke; certain pesticides; drugs, and viruses. Certain outcomes associated with development of a hyperproliferative disorder include non-malignant tumors, pre-malignant tumors, and malignant tissues in an individual. Certain risk factors and causes for development of an inflammatory condition include any noxious stimulus that causes a cellular response to an underlying pathophysiologic condition, which includes but is not limited to bacterial and viral infections, and allergens. Certain outcomes associated with development of an inflammatory condition include redness, pain, swelling at the affected area, loss of function, morbidity and mortality of the individual.

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

Detailed Description

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

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this disclosure, including, but not limited to, patents, patent applications, published patent applications, articles, books, treatises, and GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

Definitions

Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis.

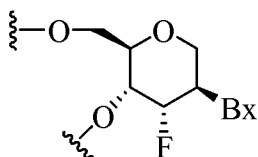
Unless otherwise indicated, the following terms have the following meanings:

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

“2'-deoxyribonucleoside” means a nucleoside comprising 2'-H furanosyl sugar moiety, as found in naturally occurring deoxyribonucleosides (DNA).

“2'-MOE nucleoside” (also 2'-O-methoxyethyl nucleoside) means a nucleoside comprising a 2'-MOE modified sugar moiety.

“3'-fluoro-HNA” (also “F-HNA” or “3'-F-HNA”) means the sugar moiety of a nucleoside having the following structure:



wherein Bx is a nucleobase.

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

“About” means within $\pm 7\%$ of a value. For example, if it is stated, “the compounds affected at least about 70% inhibition of Factor VII,” it is implied that the Factor VII levels are inhibited within a range of 63% and 77%.

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

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

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

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

“Amelioration” or “ameliorate” or “ameliorating” 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 activity.

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

5 “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.

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

“Antisense compound” means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding. Examples of antisense compounds include single-
15 stranded and double-stranded compounds, such as, antisense oligonucleotides, siRNAs, shRNAs, snoRNAs, miRNAs, and satellite repeats.

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

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

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

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

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

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

“Constrained ethyl nucleoside” (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge.

35 “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 VII nucleic acid” or “Factor 7 nucleic acid” or “F VII nucleic acid” or “F 7 nucleic acid” means any nucleic acid encoding Factor VII. For example, in certain embodiments, a Factor VII nucleic acid includes, a DNA sequence encoding Factor VII, an RNA sequence transcribed from DNA encoding Factor

VII (including genomic DNA comprising introns and exons), and an mRNA sequence encoding Factor VII. “Factor VII mRNA” means an mRNA encoding a Factor VII protein.

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

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

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

“Furanosyl” means a structure comprising a 5-membered ring comprising four carbon atoms and one oxygen atom.

“Gapmer” means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNaseH 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 external regions. The internal region may be referred to as a “gap” and the external regions may be referred to as the “wings.”

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

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

“Hyperproliferative disorder” refers to disorders characterized by an abnormal or pathological proliferation of cells, for example, cancer, psoriasis, hyperplasia and the like.

“Identifying an animal at risk for developing a hyperproliferative disorder” means identifying an animal having been diagnosed with a hyperproliferative disorder, or identifying an animal predisposed to develop a hyperproliferative disorder. Individuals predisposed to develop a hyperproliferative disorder

include those having one or more risk factors for hyperproliferative disorders including genetic factors, such as gene mutations and chromosomal aberrations, which may or may not be inherited; and environmental factors, which include, but are not limited to, exposure to known mutagens, such as high energy radiation from radioactive elements, X-rays, gamma rays, microwaves, and ultraviolet light; certain industrial chemicals; pollutants such as cigarette smoke; certain pesticides; drugs, and viruses. Such identification may be accomplished by any method including evaluating an individual's medical history and standard clinical tests or assessments.

"Identifying an animal at risk for developing an inflammatory condition" means identifying an animal having been diagnosed with an inflammatory condition, or identifying an animal predisposed to develop an inflammatory condition. Individuals predisposed to develop an inflammatory condition include those having one or more risk factors for inflammatory disorders including contact with any noxious stimulus that causes a cellular response to an underlying pathophysiologic condition, which includes but is not limited to bacterial and viral infections, and allergens. Such identification may be accomplished by any method including evaluating an individual's medical history and standard clinical tests or assessments.

"Identifying an animal at risk for developing a thromboembolic complication" 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.

"Individual in need thereof" refers to a human or non-human animal selected for treatment or therapy that is in need of such treatment or therapy.

"Inflammatory condition" refers to a disease, disease state, syndrome, or other condition resulting in inflammation. For example, rheumatoid arthritis and liver fibrosis are inflammatory conditions. Other examples of inflammatory conditions include sepsis, myocardial ischemia/reperfusion injury, adult respiratory distress syndrome, nephritis, graft rejection, inflammatory bowel disease, multiple sclerosis, arteriosclerosis, and vasculitis.

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

"ISIS 473589" means a Factor VII reducing agent that is a modified antisense oligonucleotide having the nucleobase sequence (from 5' to 3') "GCTAAACAACCGCCTT", incorporated herein as SEQ ID NO:

59, consisting of a combination of sixteen 2'-deoxyribonucleosides, MOE nucleosides, and cEt nucleosides, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage and each cytosine is a 5-

methylcytosine. From the 5' end to the 3' end, each nucleoside of ISIS 473589 has the following sugar moiety: cEt, 2'-deoxyribose, cEt, 2'-deoxyribose, cEt, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, MOE, MOE. The chemical modifications can also be represented by the formula: Gks mCds Tks Ads Aks Ads mCds Ads
 5 Ads mCds mCds Gds mCds mCds Tes Te, wherein 'k' indicates a cEt sugar moiety; 'd' indicates a deoxyribose moiety; 'e' indicates a MOE sugar moiety; 'mC' indicates a 5-methylcytosine; and 's' indicates a phosphorothioate linkage (P=S).

"ISIS 490279" means a Factor VII reducing agent that is a modified antisense oligonucleotide having the nucleobase sequence (from 5' to 3') "CCCTCCTGTGCCTGGATGCT", incorporated herein as SEQ ID
 10 NO: 93, a 5-10-5 MOE gapmer, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage and each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl moiety. The chemical modifications can also be represented by the formula: mCes mCes mCes Tes mCes mCds Tds Gds Tds Gds mCds mCds Tds Gds Gds Aes Tes Ges mCes Te, wherein 'd' indicates a deoxyribose moiety; 'e' indicates a MOE sugar moiety; 'mC' indicates a 5-methylcytosine; and 's' indicates
 15 a phosphorothioate linkage (P=S).

"ISIS 540175" means a Factor VII reducing agent that is a modified antisense oligonucleotide having the nucleobase sequence (from 5' to 3') "GGACACCCACGCCCCC", incorporated herein as SEQ ID
 NO:637, consisting of a combination of sixteen deoxynucleosides, MOE nucleosides, and cEt nucleosides, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage and each cytosine is a 5-
 20 methylcytosine. From the 5' end to the 3' end, each nucleoside of ISIS 540175 has the following sugar moiety: MOE, MOE, cEt, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, 2'-deoxyribose, cEt, cEt, MOE. The chemical modifications can also be represented by the formula: Ges Ges Aks mCds Ads mCds mCds mCds Ads mCds Gds mCds mCds mCks mCks mCe, wherein 'k' indicates a cEt sugar moiety; 'd' indicates a
 25 deoxyribose; 'e' indicates a MOE sugar moiety; 'mC' indicates a 5-methylcytosine; and 's' indicates a phosphorothioate linkage (P=S).

"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.

30 "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.

“MOE nucleoside” means a nucleoside comprising a 2'-substituted sugar moiety comprising MOE at the 2'-position.

“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.

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

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

10 “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
15 (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.

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

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

25 “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.

30 “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.

35 “Sugar moiety” means a naturally occurring sugar moiety or a modified sugar moiety of a nucleoside.

“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.

5 “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.

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

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

15 *Certain Embodiments*

Certain embodiments provide methods for decreasing Factor VII mRNA and protein expression.

Certain embodiments provide methods for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with Factor VII in an individual in need thereof. Also contemplated are
20 methods for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or conditions associated with Factor VII. Factor VII associated diseases, disorders, and conditions include thromboembolic complications, hyperproliferative disorders, and inflammatory conditions. Certain such thromboembolic complications include thrombosis, embolism, and thromboembolism, such as, deep vein thrombosis, pulmonary embolism, myocardial infarction, stroke, cancer, rheumatoid arthritis, and
25 fibrosis. Certain such hyperproliferative disorders include cancer, psoriasis, hyperplasia and the like. Certain such inflammatory conditions include rheumatoid arthritis, liver fibrosis, sepsis, myocardial ischemia/reperfusion injury, adult respiratory distress syndrome, nephritis, graft rejection, inflammatory bowel disease, multiple sclerosis, arteriosclerosis, and vasculitis.

Such diseases, disorders, and conditions can have one or more risk factors, causes, or outcomes in
30 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
35 death of the individual. Certain risk factors and causes for development of a hyperproliferative disorder include genetic factors, such as gene mutations and chromosomal aberrations, which may or may not be

inherited; and environmental factors, which include, but are not limited to, exposure to known mutagens, such as high energy radiation from radioactive elements, X-rays, gamma rays, microwaves, and ultraviolet light; certain industrial chemicals; pollutants such as cigarette smoke; certain pesticides; drugs, and viruses. Certain outcomes associated with development of a hyperproliferative disorder include non-malignant tumors, pre-malignant tumors and malignant tissues in an individual. Certain risk factors and causes for development of an inflammatory condition include any noxious stimulus that causes a cellular response to an underlying pathophysiologic condition, which includes but is not limited to bacterial and viral infections, and allergens. Inflammation is mediated by cytokines, which are secreted by the host macrophages, T-lymphocytes, endothelial cells. Certain outcomes associated with development of an inflammatory condition include redness, pain, swelling at the affected area, loss of function, morbidity and mortality of the individual.

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

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

In certain embodiments, provided herein 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 VII. Factor VII associated diseases, disorders, and conditions include thromboembolic complications, hyperproliferative disorders, and inflammatory conditions. Thromboembolic complications include thrombosis, embolism, thromboembolism, deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke. Hyperproliferative disorders include cancer. Inflammatory conditions include rheumatoid arthritis and fibrosis.

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

Embodiments described herein provide a Factor VII 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 described herein provide a Factor VII 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 described herein provide the use of a Factor VII 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 described herein provide the use of a Factor VII 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 described herein provide a Factor VII specific inhibitor, as described herein, for use in treating, preventing, or ameliorating hyperproliferative disorder such as cancer, psoriasis, and hyperplasia.

Embodiments described herein provide a Factor VII specific inhibitor, as described herein, for use in treating, preventing, or ameliorating a hyperproliferative disorder, 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 described herein provide the use of a Factor VII specific inhibitor, as described herein, in the manufacture of a medicament for treating, preventing, or ameliorating a hyperproliferative disorder, 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 described herein provide the use of a Factor VII specific inhibitor, as described herein, in the manufacture of a medicament for treating, preventing, or ameliorating a hyperproliferative disorder, as described herein, in a patient who is subsequently administered an additional agent or therapy, as described herein.

Embodiments described herein provide a Factor VII specific inhibitor, as described herein, for use in treating, preventing, or ameliorating inflammatory conditions such as rheumatoid arthritis, liver fibrosis, sepsis, myocardial ischemia/reperfusion injury, adult respiratory distress syndrome, nephritis, graft rejection, inflammatory bowel disease, multiple sclerosis, arteriosclerosis, and vasculitis.

Embodiments described herein provide a Factor VII specific inhibitor, as described herein, for use in treating, preventing, or ameliorating an inflammatory condition, 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 described herein provide the use of a Factor VII specific inhibitor, as described herein, in the manufacture of a medicament for treating, preventing, or ameliorating an inflammatory condition, 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 described herein provide the use of a Factor VII specific inhibitor, as described herein, in the manufacture of a medicament for treating, preventing, or ameliorating an inflammatory condition, as described herein, in a patient who is subsequently administered an additional agent or therapy, as described herein.

In certain embodiments, Factor VII specific inhibitors are peptides or proteins, such as, but not limited to, GP 1-49 (Martin, D.M. et al., Biochemistry. 1993. 32: 13949-13955); peptide-(285-305), peptide-(44-50), peptide-(194-214), peptide-(208-229), and peptide-(376-390) (Kumar, A. et al., J. Biol. Chem. 1991. 266: 915-921); modified Factor VII (USPN 5,824,639); and modified Factor VII (USPPN 2004/0197370).

5 In certain embodiments, Factor VII specific inhibitors are antibodies, such as, but not limited to, GP 1-49 (Martin, D.M. et al., Biochemistry. 1993. 32: 13949-13955); peptide-(285-305), peptide-(44-50), peptide-(194-214), peptide-(208-229), and peptide-(376-390) (Kumar, A. et al., J. Biol. Chem. 1991. 266: 915-921); modified Factor VII (USPN 5,824,639); and modified Factor VII (USPPN 2004/0197370).

10 In certain embodiments, Factor VII specific inhibitors are small molecules, such as, but not limited to, curcumin (Koizume, S. et al., Mol. Cancer Res. 2009. 7: 1928-1936); thrombin (Hultin, M.B. and Jesty, J. Blood 1981. 57: 476-482); phospholipase C Hubbard A.R. and Parr, L.J. Br. J. Haematol. 1989. 73: 360-364); ruthenium red (Chu, A.J. et al; Br. J. Pharmacol. 2001. 133: 659-664); and 1-hydroxy-7-hydroxycarbamoylquinoxaline-2,3(1H,4H)-dione compounds (USPN 5,859,010).

15 In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 16, at least 18, at least 19, or at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1381 to 1406 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

20 In certain embodiments, the modified oligonucleotide consists of 15 to 30, 18 to 24, 19 to 22, or 20 linked nucleosides.

25 In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 15128 to 15150 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

30 In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2592 to 2607, 2626 to 2641, 2660 to 2675, 2796 to 2811, 2966 to 2981, 3000 to 3015, 3034 to 3049, 3068 to 3083, 3153 to 3168, 3170 to 3185, 3272 to 3287, 3374 to 3389, 3578 to 3593, 3851 to 3866, 3953 to 3968, 4124 to 4139, 4260 to 4275, 4311 to 4326, 4447 to 4462, and 4532 to 4547 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

35 In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at

least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2592 to 2607, 2626 to 2641, 2660 to 2675, 2796 to 2811, 2966 to 2981, 3000 to 3015, 3034 to 3049, 3068 to 3083, 3153 to 3168, 3170 to 3185, 3272 to 3287, 3374 to 3389, 3578 to 3593, 3851 to 3866, 3953 to 3968, 4124 to 4139, 4260 to 4275, 4311 to 4326, 4447 to 4462, or 4532 to 4547 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 1387 to 1406, 15128 to 15143, 15192 to 15207, and 15131 to 15146 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2692 to 2707, 2760 to 2775, 2862 to 2877, 2930 to 2945, 3117 to 3132, 3338 to 3353, 3440 to 3455, 3508 to 3523, 3542 to 3557, 3628 to 3643, 3662 to 3677, 3781 to 3796, 3815 to 3830, 3917 to 3932, 4190 to 4205, 4224 to 4239, 4377 to 4392, and/or 4411 to 4426 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 3109 to 3124, 3194 to 3209, 3330 to 3345, 3432 to 3447, 3500 to 3515, 3534 to 3549, 3620 to 3635, 3654 to 3669, 3773 to 3788, 4182 to 4197, 4216 to 4231, 4369 to 4384, and/or 4403 to 4418 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2565 to 2580, 2633 to 2648, 2667 to 2682, 2735 to 2750, 2803 to 2818, 2837 to 2852, 2905 to 2920, 3007 to 3022, 3041 to 3056, 3075 to 3090, 3092 to 3107, 3279 to 3294, 3381 to 3396, 3483 to 3498, 3603 to 3618, 3722 to 3737, 3756 to 3771, 3858 to 3873, 3892 to 3907, 3960 to 3975, 4046 to 4061, 4131 to 4146, 4165 to 4180, 4318 to 4333, and/or 4454 to 4469 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2558 to 4600 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 15128 to 15150, 15181 to 15224, 15128 to 15150, 2560 to 2609, 2684 to 2717, or 3103 to 3131 of SEQ ID NO: 1. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

In certain embodiments, the modified oligonucleotide consists of 13 to 25, 14 to 25, 15 to 25, or 16 linked nucleosides.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to SEQ ID NO: 1.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 59.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 16, at least 18, at least 19, or at least 20 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 93.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 637.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of any of the nucleobase sequences of SEQ IDNO: 59, 93, 259, 254, 624, 637, 644, or 653.

In certain embodiments, provided herein are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NO: 21-559.

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

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

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

5 In certain embodiments, the modified oligonucleotide comprises at least one modified sugar. In certain embodiments, the modified sugar is any of a 2'-O-methoxyethyl, a constrained ethyl, or a 3'-fluoro-HNA.

In certain embodiments, the compound comprises at least one 2'-O-methoxyethyl nucleoside, a constrained ethyl nucleoside, or a 3'-fluoro-HNA nucleoside.

10 In certain embodiments, provided herein are compounds comprising a modified oligonucleotide according to the following formula:

Gks mCds Tks Ads Aks Ads mCds Ads Ads mCds mCds Gds mCds mCds Tes Te; wherein, each nucleobase is indicated according to the following:

A = adenine

15 T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

20 d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

25 In certain embodiments, provided herein are compounds consisting of a modified oligonucleotide according to the following formula:

Gks mCds Tks Ads Aks Ads mCds Ads Ads mCds mCds Gds mCds mCds Tes Te; wherein, each nucleobase is indicated according to the following:

A = adenine

T = thymine

30 G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

35 e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

In certain embodiments, provided herein are compounds comprising of a modified oligonucleotide according to the following formula:

mCes mCes mCes Tes mCes mCds Tds Gds Tds Gds mCds mCds Tds Gds Gds Aes Tes Ges mCes Te;

5 wherein,

each nucleobase is indicated according to the following:

A = adenine

T = thymine

G = guanine;

10 mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

15 each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

In certain embodiments, provided herein are compounds consisting of a modified oligonucleotide according to the following formula:

mCes mCes mCes Tes mCes mCds Tds Gds Tds Gds mCds mCds Tds Gds Gds Aes Tes Ges mCes Te;

20 wherein,

each nucleobase is indicated according to the following:

A = adenine

T = thymine

G = guanine;

25 mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

30 each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

In certain embodiments, provided herein are compounds comprising of a modified oligonucleotide according to the following formula:

Ges Ges Aks mCds Ads mCds mCds mCds Ads mCds Gds mCds mCds mCks mCks mCe; wherein,

35 each nucleobase is indicated according to the following:

A = adenine

T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

5 k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

10 In certain embodiments, provided herein are compounds consisting of a modified oligonucleotide according to the following formula:

Ges Ges Aks mCds Ads mCds mCds mCds Ads mCds Gds mCds mCds mCks mCks mCe; wherein,

each nucleobase is indicated according to the following:

A = adenine

15 T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

20 d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

25 In certain embodiments, provided herein are compositions comprising a compound as described herein or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, provided herein are compounds and compositions as described herein for use in therapy.

In certain embodiments, provided herein are compounds and compositions as described herein for use in treating, preventing, or slowing progression of a thromboembolic complication.

30 In certain embodiments, provided herein are compounds and compositions as described herein for use in treating, preventing, or slowing progression of a hyperproliferative disorder.

In certain embodiments, provided herein are compounds and compositions as described herein for use in treating, preventing, or slowing progression of an inflammatory condition.

35 *Antisense Compounds*

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

5 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.

10 In certain embodiments, an antisense compound targeted to a Factor VII 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, 15 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleosides.

In certain embodiments, antisense oligonucleotides targeted to a Factor VII nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or 20 alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a Factor VII 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.

25 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 30 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 35 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 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to a Factor VII nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties, such as enhanced 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 a substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may, in some embodiments, include β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE, and 2'-O-CH₃, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH₂)_n-O-2' bridge, where n=1 or n=2). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5' wing

region, “Y” represents the length of the gap region, and “Z” represents the length of the 3’ wing region. As used herein, a gapmer described as “X-Y-Z” has a configuration such that the gap segment is positioned immediately adjacent each of the 5’ wing segment and the 3’ wing segment. Thus, no intervening nucleotides exist between the 5’ wing segment and gap segment, or the gap segment and the 3’ wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, or more nucleotides. Thus, gapmers described herein 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 or 2-8-2.

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 described herein include, but are not limited to, for example, 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, or 5-13.

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

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

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

In certain embodiments, antisense compounds targeted to a Factor VII nucleic acid possess a 2-12-2 gapmer motif.

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

In certain embodiments, a gap-widened antisense oligonucleotide targeted to a Factor VII nucleic acid has a gap segment of fourteen 2’-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 VII 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.

In certain embodiments, the compounds or compositions comprise modified oligonucleotides consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising a portion at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleobases

complementary to an equal length portion of any one of the nucleobase ranges: 1381 to 1406, 15128 to 15150, 1387 to 1406, 15128 to 15143, 15192 to 15207, 15131 to 15146, 2592 to 2607, 2626 to 2641, 2660 to 2675, 2796 to 2811, 2966 to 2981, 3000 to 3015, 3034 to 3049, 3068 to 3083, 3153 to 3168, 3170 to 3185, 3272 to 3287, 3374 to 3389, 3578 to 3593, 3851 to 3866, 3953 to 3968, 4124 to 4139, 4260 to 4275, 4311 to 4326, 4447 to 4462, 4532 to 4547, 2692 to 2707, 2760 to 2775, 2862 to 2877, 2930 to 2945, 3117 to 3132, 3338 to 3353, 3440 to 3455, 3508 to 3523, 3542 to 3557, 3628 to 3643, 3662 to 3677, 3781 to 3796, 3815 to 3830, 3917 to 3932, 4190 to 4205, 4224 to 4239, 4377 to 4392, 4411 to 4426, 3109 to 3124, 3194 to 3209, 3330 to 3345, 3432 to 3447, 3500 to 3515, 3534 to 3549, 3620 to 3635, 3654 to 3669, 3773 to 3788, 4182 to 4197, 4216 to 4231, 4369 to 4384, 4403 to 4418, 2565 to 2580, 2633 to 2648, 2667 to 2682, 2735 to 2750, 2803 to 2818, 2837 to 2852, 2905 to 2920, 3007 to 3022, 3041 to 3056, 3075 to 3090, 3092 to 3107, 3279 to 3294, 3381 to 3396, 3483 to 3498, 3603 to 3618, 3722 to 3737, 3756 to 3771, 3858 to 3873, 3892 to 3907, 3960 to 3975, 4046 to 4061, 4131 to 4146, 4165 to 4180, 4318 to 4333, 4454 to 4469, 2558 to 4600, 15128 to 15150, 15181 to 15224, 15128 to 15150, 2560 to 2609, 2684 to 2717, and/or 3103 to 3131 wherein the nucleobase sequence is complementary to SEQ ID NO: 1. In certain embodiments, such oligonucleotides have a gap segment of 9, 10, or more linked deoxynucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1, 2, 3, 4, or 5 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified nucleoside is a constrained ethyl (cEt) nucleoside. In certain embodiments, the modified nucleoside is a F-HNA nucleoside. In certain embodiments, each modified nucleoside in each wing segment is independently a 2'-MOE nucleoside or a nucleoside with a bicyclic sugar modification such as a constrained ethyl (cEt) nucleoside or LNA nucleoside. In certain embodiments, each modified nucleoside in each wing segment is independently a 2'-MOE nucleoside, a nucleoside with a bicyclic sugar modification such as a constrained ethyl (cEt) nucleoside or LNA nucleoside, or a 2'-deoxyribonucleoside.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 21-559. In certain embodiments, such oligonucleotides have a gap segment of 8, 9, 10, or more linked deoxynucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1, 2, 3, 4, 5, 6, 7, or 8 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include

nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified nucleoside is a constrained ethyl (cEt) nucleoside. In certain embodiments, the modified nucleoside is a F-HNA nucleoside. In certain embodiments, each modified nucleoside in each wing segment is independently a 2'-MOE nucleoside, a nucleoside with a bicyclic sugar modification such as a constrained ethyl (cEt) nucleoside or LNA nucleoside, or a 2'-deoxyribonucleoside.

In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 linked nucleosides. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1, 2, 3, 4, or 5 sugar modified nucleosides. In certain embodiments, each sugar modified nucleoside is independently a 2'-MOE nucleoside, a nucleoside with a bicyclic sugar moiety such as a constrained ethyl (cEt) nucleoside or LNA nucleoside, or a F-HNA nucleoside. In certain embodiments, each modified nucleoside in each wing segment is independently a 2'-MOE nucleoside, a nucleoside with a bicyclic sugar modification such as a constrained ethyl (cEt) nucleoside or LNA nucleoside, a 2'-deoxyribonucleoside, or a F-HNA nucleoside.

In certain embodiments, the compounds or compositions comprise a salt of the modified oligonucleotide.

In certain embodiments, the modified oligonucleotide comprises: a) a gap segment consisting of linked deoxynucleosides; b) a 5' wing segment consisting of linked nucleosides; and c) a 3' wing segment consisting of linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment and each nucleoside of each wing segment comprises a modified sugar.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 10 linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar and/or a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine. In some aspects, each of the three linked nucleosides of the 5' wing segment is a 2'-O-methoxyethyl nucleoside and each of the three linked nucleosides of the 3' wing segment is a constrained ethyl (cEt) nucleoside. In other aspects, the three linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl nucleoside, a constrained ethyl (cEt) nucleoside, and a constrained ethyl (cEt) nucleoside in the 5' to 3' direction, and the three linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) nucleoside, a constrained ethyl (cEt) nucleoside, and a 2'-O-methoxyethyl nucleoside in the 5' to 3' direction. In other aspects, the three linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl nucleoside, 2'-O-methoxyethyl nucleoside, and a constrained ethyl (cEt) nucleoside in the 5' to 3' direction, and the three linked nucleosides of the 3' wing segment are a

constrained ethyl (cEt) nucleoside, a constrained ethyl (cEt) nucleoside, and a 2'-O-methoxyethyl nucleoside in the 5' to 3' direction.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 10 linked deoxynucleosides, the 5' wing segment consisting of one nucleoside, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar and/or a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine. In some aspects, the nucleoside of the 5' wing segment is a constrained ethyl (cEt) nucleoside and the five linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) nucleoside, 2'-O-methoxyethyl nucleoside, a constrained ethyl (cEt) nucleoside, a 2'-O-methoxyethyl nucleoside, and a 2'-O-methoxyethyl nucleoside in the 5' to 3' direction.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 9 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of two linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, a 2'-deoxyribose, and/or a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine. In some aspects, the five linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) nucleoside, a 2'-deoxynucleoside, a constrained ethyl (cEt) nucleoside, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar and the two linked nucleosides of the 3' wing segment are a 2'-O-methoxyethyl nucleoside and a 2'-O-methoxyethyl sugar in the 5' to 3' direction.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any one of the nucleobase ranges: 1381 to 1406, 15128 to 15150, 1387 to 1406, 15128 to 15143, 15192 to 15207, 15131 to 15146, 2592 to 2607, 2626 to 2641, 2660 to 2675, 2796 to 2811, 2966 to 2981, 3000 to 3015, 3034 to 3049, 3068 to 3083, 3153 to 3168, 3170 to 3185, 3272 to 3287, 3374 to 3389, 3578 to 3593, 3851 to 3866, 3953 to 3968, 4124 to 4139, 4260 to 4275, 4311 to 4326, 4447 to 4462, 4532 to 4547, 2692 to 2707, 2760 to 2775, 2862 to 2877, 2930 to 2945, 3117 to 3132, 3338 to 3353, 3440 to 3455, 3508 to 3523, 3542 to 3557, 3628 to 3643, 3662 to 3677, 3781 to 3796, 3815 to 3830, 3917 to 3932, 4190 to 4205, 4224 to 4239, 4377 to 4392, 4411 to 4426, 3109 to 3124, 3194 to 3209, 3330 to 3345, 3432 to 3447, 3500 to 3515, 3534 to 3549, 3620 to 3635, 3654 to 3669, 3773 to 3788, 4182 to 4197, 4216 to 4231, 4369 to 4384, 4403 to 4418, 2565 to 2580, 2633 to 2648, 2667 to 2682, 2735 to 2750, 2803 to 2818, 2837 to 2852, 2905 to 2920, 3007 to 3022, 3041 to 3056, 3075 to 3090, 3092 to 3107, 3279 to 3294, 3381 to 3396, 3483 to 3498, 3603 to 3618, 3722 to 3737, 3756 to 3771, 3858 to 3873, 3892 to 3907, 3960 to 3975, 4046 to 4061, 4131 to 4146, 4165 to 4180, 4318 to 4333, 4454 to 4469, 2558 to 4600, 15128 to 15150, 15181 to 15224, 15128 to 15150, 2560 to 2609, 2684 to 2717, and/or 3103 to 3131 wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment

consisting of three linked nucleosides; and c) a 3' wing segment consisting of three linked nucleosides. In some aspects, the gap segment is positioned between the 5' wing segment and the 3' wing segment; each of the three linked nucleosides of the 5' wing segment is a 2'-O-methoxyethyl sugar and each of the three linked nucleosides of the 3' wing segment is a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine residue is a 5-methylcytosine. In other aspects, the gap segment is positioned between the 5' wing segment and the 3' wing segment; the three linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any one of the nucleobase ranges: 1381 to 1406, 15128 to 15150, 1387 to 1406, 15128 to 15143, 15192 to 15207, 15131 to 15146, 2592 to 2607, 2626 to 2641, 2660 to 2675, 2796 to 2811, 2966 to 2981, 3000 to 3015, 3034 to 3049, 3068 to 3083, 3153 to 3168, 3170 to 3185, 3272 to 3287, 3374 to 3389, 3578 to 3593, 3851 to 3866, 3953 to 3968, 4124 to 4139, 4260 to 4275, 4311 to 4326, 4447 to 4462, 4532 to 4547, 2692 to 2707, 2760 to 2775, 2862 to 2877, 2930 to 2945, 3117 to 3132, 3338 to 3353, 3440 to 3455, 3508 to 3523, 3542 to 3557, 3628 to 3643, 3662 to 3677, 3781 to 3796, 3815 to 3830, 3917 to 3932, 4190 to 4205, 4224 to 4239, 4377 to 4392, 4411 to 4426, 3109 to 3124, 3194 to 3209, 3330 to 3345, 3432 to 3447, 3500 to 3515, 3534 to 3549, 3620 to 3635, 3654 to 3669, 3773 to 3788, 4182 to 4197, 4216 to 4231, 4369 to 4384, 4403 to 4418, 2565 to 2580, 2633 to 2648, 2667 to 2682, 2735 to 2750, 2803 to 2818, 2837 to 2852, 2905 to 2920, 3007 to 3022, 3041 to 3056, 3075 to 3090, 3092 to 3107, 3279 to 3294, 3381 to 3396, 3483 to 3498, 3603 to 3618, 3722 to 3737, 3756 to 3771, 3858 to 3873, 3892 to 3907, 3960 to 3975, 4046 to 4061, 4131 to 4146, 4165 to 4180, 4318 to 4333, 4454 to 4469, 2558 to 4600, 15128 to 15150, 15181 to 15224, 15128 to 15150, 2560 to 2609, 2684 to 2717, and/or 3103 to 3131 wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of two linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. In some aspects, the gap segment is positioned between the 5' wing segment and the 3' wing segment; the two linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, 2'-O-methoxyethyl sugar, constrained ethyl (cEt) sugar, and 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the antisense compounds targeted to a Factor VII nucleic acid has any of the following sugar motifs:

k-d(10)-k
 e-d(10)-k
 k-d(10)-e
 k-k-d(10)-k-k
 5 k-k-d(10)-e-e
 e-e-d(10)-k-k
 k-k-k-d(10)-k-k-k
 e-e-e-d(10)-k-k-k
 k-k-k-d(10)-e-e-e
 10 k-k-k-d(10)-k-k-k
 e-k-k-d(10)-k-k-e
 e-e-k-d(10)-k-k-e
 e-d-k-d(10)-k-k-e
 e-k-d(10)-k-e-k-e
 15 k-d(10)-k-e-k-e-e
 e-e-k-d(10)-k-e-k-e
 e-d-d-k-d(9)-k-k-e
 e-e-e-e-d(9)-k-k-e
 e-e-e-e-d(10)-e-e-e-e-e
 20 k-d-k-d-k-d(9)-e-e
 k-d(10)-k-e-k-e-e

wherein, 'k' is a constrained ethyl nucleoside, 'e' is a 2'-MOE substituted nucleoside, and 'd' is a 2'-deoxynucleoside. Other motifs and modifications may be applied to the sequences described herein,

including those motifs and modifications described in USSN 61/440,828 filed on 2/8/2011, USSN
 25 61/470,927 filed on 4/1/2011, and CORE0094WO filed concurrently herewith, all entitled "OLIGOMERIC
 COMPOUNDS COMPRISING BICYCLIC NUCLEOTIDES AND USES THEREOF" and USSN
 61/522,659 filed on 08/11/2011 and CORE0099US.L2 filed concurrently herewith, both entitled
 "SELECTIVE ANTISENSE COMPOUNDS AND USES THEREOF," all of which are incorporated herein
 by reference.

30

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode the Factor VII gene sequence include, without limitation, the
 following: GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000,
 incorporated herein as SEQ ID NO: 1; GENBANK Accession No. NM_019616.2, incorporated herein as
 35 SEQ ID NO: 2; DB184141.1, designated herein as SEQ ID NO: 3; and GENBANK Accession No.
 NW_001104507.1 truncated from nucleotides 691000 to 706000, designated herein as SEQ ID NO: 4.

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
5 (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, a translation termination region, or other defined nucleic acid region. The structurally defined regions for Factor VII can be obtained by accession number from sequence
10 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
15 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
20 embodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5
25 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifically exclude a certain structurally defined region,
30 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
35 selected target nucleic acid (i.e., non-target or off-target sequences).

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

Hybridization

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

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

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

Complementarity

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

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

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

determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656). Percent homology, sequence identity or complementarity can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a Factor VII nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and/or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

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

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

10 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
15 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.

20 *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
25 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
30 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.

35 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.

5 *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.

10 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
15 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.

20

Modified Internucleoside Linkages

The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages
25 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,
30 phosphodiester, phosphotriester, methylphosphonate, phosphoramidate, and phosphorothioate. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a Factor 12 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
35 is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substituent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or C(R₁)(R₂) (R, R₁ and R₂ are each independently 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, 2'-OCH₂CH₃, 2'-OCH₂CH₂F 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, OCH₂F, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(R_m)(R_n), O-CH₂-C(=O)-N(R_m)(R_n), and O-CH₂-C(=O)-N(R₁)-(CH₂)₂-N(R_m)(R_n), where each R₁, R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' and 4'-CH(CH₂OCH₃)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (see Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

Further reports related to bicyclic nucleosides can also be found in published literature (see for example: Singh *et al.*, *Chem. Commun.*, 1998, 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630;

Wahlestedt *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava *et al.*, *J. Am. Chem. Soc.*, 2007, 129(26) 8362-8379; Elayadi *et al.*, *Curr. Opinion Invest. Drugs*, 2001, 2, 558-561; Braasch *et al.*, *Chem. Biol.*, 2001, 8, 1-7; and Orum *et al.*, *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 7,034,133; 7,053,207; 7,399,845; 7,547,684; and 7,696,345; U.S. Patent Publication No. US2008-0039618; US2009-0012281; U.S. Patent Serial Nos. 60/989,574; 61/026,995; 61/026,998; 61/056,564; 61/086,231; 61/097,787; and 61/099,844; Published PCT International applications WO 1994/014226; WO 2004/106356; WO 2005/021570; WO 2007/134181; WO 2008/150729; WO 2008/154401; and WO 2009/006478. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

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

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

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

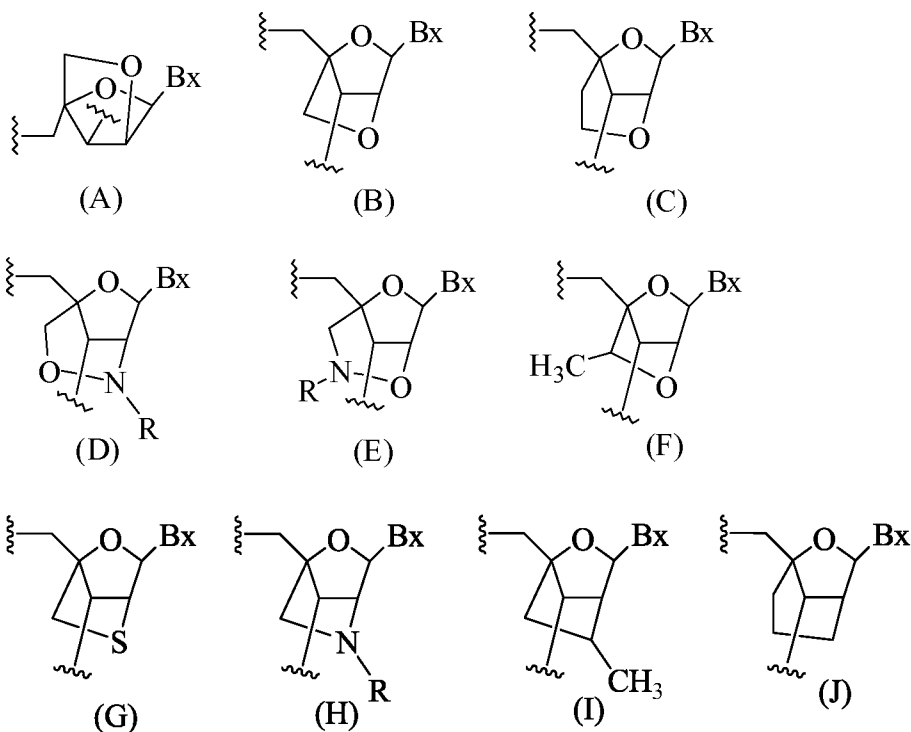
each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)$ -H), substituted acyl, a heterocycle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

In certain embodiments, the bridge of a bicyclic sugar moiety is $-[C(R_a)(R_b)]_n$ -, $-[C(R_a)(R_b)]_n-O$ -, $-C(R_aR_b)-N(R)-O$ - or $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R)-2' and 4'-CH₂-N(R)-O-2' wherein each R is, independently, H, a protecting group or C_1 - C_{12} alkyl.

In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α -L configuration or in the β -D configuration. Previously, α -L-methyleneoxy (4'-CH₂-O-2') BNA's have been incorporated into antisense

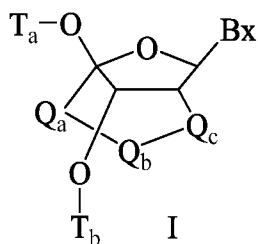
oligonucleotides that showed antisense activity (Frieden *et al.*, *Nucleic Acids Research*, 2003, 21, 6365-6372).

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α -L-methyleneoxy (4'-CH₂-O-2') BNA, (B) β -D-methyleneoxy (4'-CH₂-O-2') BNA, (C) ethyleneoxy (4'-(CH₂)₂-O-2') BNA, (D) aminooxy (4'-CH₂-O-N(R)-2') BNA, (E) oxyamino (4'-CH₂-N(R)-O-2') BNA, and (F) methyl(methyleneoxy) (4'-CH(CH₃)-O-2') BNA, (G) methylene-thio (4'-CH₂-S-2') BNA, (H) methylene-amino (4'-CH₂-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH₂-CH(CH₃)-2') BNA, and (J) propylene carbocyclic (4'-(CH₂)₃-2') BNA as depicted below.



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

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



wherein:

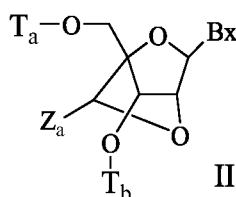
Bx is a heterocyclic base moiety;

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

R_c is C_1 - C_{12} alkyl or an amino protecting group; and

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

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



wherein:

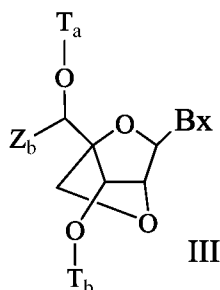
Bx is a heterocyclic base moiety;

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

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

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

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



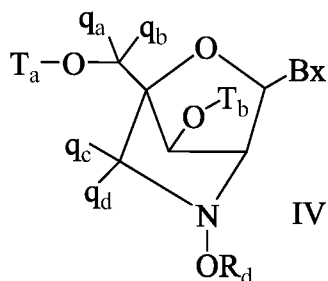
wherein:

Bx is a heterocyclic base moiety;

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

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

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



wherein:

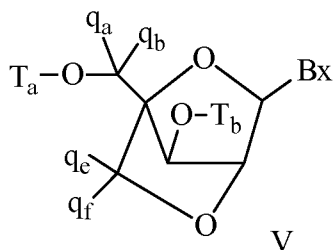
Bx is a heterocyclic base moiety;

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

R_d is C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

10 each q_a, q_b, q_c and q_d is, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, acyl, substituted acyl, C₁-C₆ aminoalkyl or substituted C₁-C₆ aminoalkyl;

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



15 wherein:

Bx is a heterocyclic base moiety;

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

20 q_a, q_b, q_c and q_d are each, independently, hydrogen, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k or N(H)C(=S)NJ_jJ_k;

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

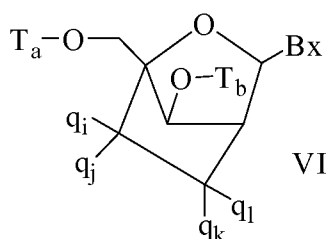
q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl or substituted C₁-C₁₂ alkyl.

25 The synthesis and preparation of the methyleneoxy (4'-CH₂-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid

recognition properties have been described (Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogues of methyleneoxy (4'-CH₂-O-2') BNA and 2'-thio-BNAs, have also been prepared (Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel *et al.*, WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel conformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

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



wherein:

Bx is a heterocyclic base moiety;

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

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

q_i and q_j or q_l and q_k together are =C(q_g)(q_h), wherein q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl or substituted C₁-C₁₂ alkyl.

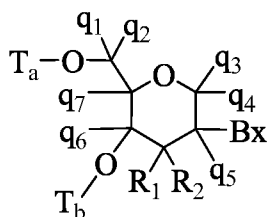
One carbocyclic bicyclic nucleoside having a 4'-(CH₂)₃-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH₂-2' have been described (Freier *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4429-4443 and Albaek *et al.*, *J. Org. Chem.*, 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic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

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

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

As used herein, “2’-modified sugar” means a furanosyl sugar modified at the 2’ position. In certain
 5 embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2’ modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$,
 10 $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2’-substituent groups can also be selected from: C_1 - C_{12} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, F, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for
 15 improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modified nucleosides comprise a 2’-MOE side chain (Baker *et al.*, *J. Biol. Chem.*, 1997, 272, 11944-12000). Such 2’-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2’-O-methyl, O-propyl, and O-aminopropyl. Oligonucleotides having the 2’-MOE substituent also have been
 20 shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, *Helv. Chim. Acta*, 1995, 78, 486-504; Altmann *et al.*, *Chimia*, 1996, 50, 168-176; Altmann *et al.*, *Biochem. Soc. Trans.*, 1996, 24, 630-637; and Altmann *et al.*, *Nucleosides Nucleotides*, 1997, 16, 917-926).

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



VII

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

Bx is a heterocyclic base moiety;

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

q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl; and each of R₁ and R₂ is selected from hydrogen, hydroxyl, halogen, substituted or unsubstituted alkoxy, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ₃C(=X)NJ₁J₂ and CN, wherein X is O, S or NJ₁ and each J₁, J₂ and J₃ is, independently, H or C₁-C₆ alkyl.

In certain embodiments, the modified THP nucleosides of Formula VII are provided wherein q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is other than H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is methyl. In certain embodiments, THP nucleosides of Formula VII are provided wherein one of R₁ and R₂ is fluoro. In certain embodiments, R₁ is fluoro and R₂ is H; R₁ is methoxy and R₂ is H, and R₁ is H and R₂ is methoxyethoxy.

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

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

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

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

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides.

In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (see for example review article: Leumann, *Bioorg. Med. Chem.*, 2002, 10, 841-854).

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

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

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

In certain embodiments, antisense compounds comprise one or more nucleosides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleosides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a bicyclic nucleoside having a (4'-CH(CH₃)-O-2') bridging group. In certain embodiments, the (4'-CH(CH₃)-O-2') modified nucleosides are arranged throughout the wings of a gapmer motif.

10 *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.

15 An antisense compounds targeted to a Factor VII nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes 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
20 a Factor VII nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an
25 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.

30 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

35 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 VII nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commercial vendors (e.g. American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, 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 VII nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitative real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA, and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT real-time PCR reagents are obtained from Invitrogen (Carlsbad, CA). RT and 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
5 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 VII nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as
10 PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of Factor VII nucleic acids can be assessed by measuring Factor VII protein levels. Protein levels of Factor VII can be evaluated or quantitated in a variety of ways well known in the art,
15 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
20 polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of mouse, rat, monkey, and human Factor VII are commercially available.

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their
25 ability to inhibit expression of Factor VII and produce phenotypic changes, such as, prolonged PT, prolonged aPTT time, decreased quantity of Platelet Factor 4 (PF-4), reduced formation of thrombus or increased time for thrombus formation, and reduction of cellular proliferation. 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
30 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 VII nucleic acid expression are measured. Changes in Factor VII protein levels are also measured using a
35 thrombin generation assay. In addition, effects on clot times, e.g. PT and aPTT, are determined using plasma from treated animals.

Certain Indications

In certain embodiments, the invention provides methods of treating an individual comprising administering one or more pharmaceutical compositions described herein. 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, infarction, 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 anti-coagulation 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 atrial fibrillation to prevent stroke. In certain embodiments the invention provides methods for prophylactically reducing Factor VII expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to a Factor VII nucleic acid.

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

In certain embodiments, the binding of Factor VII with Tissue factor to form Tissue Factor-Factor VIIa complex may lead to inflammatory conditions, such as liver fibrosis and rheumatoid arthritis and/or hyperproliferative disorders such as tumor growth and metastasis.

In certain embodiments, the individual has an inflammatory condition leading to a fibrosis complication. In certain embodiments, the individual is at risk of an excessive collagen deposition and fibrosis disorder, including, but not limited to, liver fibrosis, arterial sclerosis, chronic glomerulonephritis, cutis keloid formation, progressive systemic sclerosis (PSS), liver fibrosis, pulmonary fibrosis, cystic fibrosis, chronic graft versus host disease, scleroderma (local and systemic), Peyronie's disease, penis fibrosis, urethrostenosis after the test using a cystoscope, inner accretion after surgery, myelofibrosis, idiopathic retroperitoneal fibrosis. In certain embodiments, the individual has been identified as in need of anti-fibrotic therapy. This includes individuals with a genetic or acquired problem, disease, or disorder that leads to a risk of fibrosis, for example, α 1-antitrypsin deficiency, copper storage disease (Wilson's disease), fructosemia, galactosemia, glycogen storage diseases (such as, types II, IV, VI, IX, and X), iron overload syndromes (such as, hemochromatosis), lipid abnormalities (such as, Gaucher's disease), peroxisomal disorders (such as, Zellweger syndrome), Tyrosinemia, congenital hepatic fibrosis, bacterial infection (such as, brucellosis), parasitic infection (such as, echinococcosis), viral infections (such as, chronic hepatitis B, C), disorders

affecting hepatic blood flow (such as, Budd Chiari syndrome, heart failure, hepatic veno-occlusive disease, and portal vein thrombosis), alcohol, and drugs (such as amiodarone, chlorpromazine, Isoniazid, Methotrexate, Methyl dopa, Oxyphenisatin, and Tolbutamide). In certain embodiments, the individual has been identified as in need of anti-fibrotic therapy. In such embodiments, the tissue factor-Factor VIIa (TF/F7a) complex is identified to have the major procoagulant activity in fibrosis. In certain embodiments, the invention provides methods for prophylactically reducing Factor VII expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to a Factor VII nucleic acid.

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

In certain embodiments, the individual has an inflammatory rheumatoid arthritic complication. In certain embodiments, the individual is at risk for inflammation at the joints and rheumatoid arthritis. In such embodiments, the individual suffers from pain, swelling and tenderness at the joints, fatigue, lack of appetite, low-grade fever, muscle aches and stiffness. In certain embodiments, the individual has been identified as in need of anti-inflammatory arthritic therapy. This includes individuals suffering from rheumatoid arthritis, reactive arthritis, Reiter's syndrome, psoriatic arthritis, ankylosing spondylitis, and arthritis associated with inflammatory bowel disease. In certain embodiments, the individual has been identified as in need of anti-inflammatory therapy. In such embodiments, the tissue factor-Factor VIIa (TF/F7a) complex is identified to have the major procoagulant activity in inducing arthritis. In certain embodiments the invention provides methods for prophylactically reducing Factor VII expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to a Factor VII nucleic acid.

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

In certain embodiments, the individual has a malignant complication. In certain embodiments, the individual is at risk for tumor growth, angiogenesis and metastasis. In such embodiments, the individual suffering from hemostatic abnormalities, such as disseminated intravascular coagulation and venous thromboembolism, may suffer additional complications, such as primary and metastatic tumor growths. In such embodiments, the seeding of tumor metastases is a coagulation-dependent process. In such embodiments, the tissue factor-Factor VIIa (TF/F7a) complex is identified to have the major procoagulant activity in cancer. In certain embodiments, the individual has been identified as in need of anti- TF/F7a therapy. In certain embodiments the invention provides methods for prophylactically reducing Factor VII expression in an individual. Certain embodiments include treating an individual in need thereof by

administering to an individual a therapeutically effective amount of an antisense compound targeted to a Factor VII nucleic acid.

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

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

In certain embodiments, administration of an antisense compound targeted to a Factor VII nucleic acid results in reduction of Factor VII expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to a Factor VII nucleic acid results in a change in 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 VII 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 VII 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 VII 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 described herein 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 described herein. 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 described herein. In certain embodiments, such one or more other pharmaceutical agents are designed to treat an undesired side effect of one or more pharmaceutical compositions described herein. In certain embodiments, one or more pharmaceutical compositions described herein 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 described herein

are co-administered with another pharmaceutical agent to produce a combinational effect. In certain embodiments, one or more pharmaceutical compositions described herein are co-administered with another pharmaceutical agent to produce a synergistic effect.

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

In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition described herein include anticoagulant or antiplatelet agents. In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition described herein include, but are not limited to aspirin, clopidogrel, dipyridamole, ticlopidine, warfarin (and related
15 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, the anticoagulant or antiplatelet agent is administered prior to administration of a pharmaceutical composition described herein. In certain
20 embodiments, the anticoagulant or antiplatelet agent is administered following administration of a pharmaceutical composition described herein. In certain embodiments the anticoagulant or antiplatelet agent is administered at the same time as a pharmaceutical composition described herein. 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
25 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
30 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 first compound is an antisense compound. In certain embodiments, the second compound is an antisense
35 compound.

In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical

composition described herein include anti-inflammatory agents. In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition described herein include, but are not limited to serine protease inhibitor C1-INH recombinant protein, kallikrein antisense oligonucleotide, CINRYZE, BERINERT, KALBITOR, Icatibant, Ecallantide, attenuated androgens, anabolic steroids, and antifibrinolytic agents (e.g., epsilon-aminocaproic acid and tranexamic acid). In certain embodiments, the anti-inflammatory agent is administered prior to administration of a pharmaceutical composition described herein. In certain embodiments, the anti-inflammatory agent is administered following administration of a pharmaceutical composition described herein. In certain embodiments the anti-inflammatory agent is administered at the same time as a pharmaceutical composition described herein. In certain embodiments the dose of a co-administered anti-inflammatory agent is the same as the dose that would be administered if the anti-inflammatory agent was administered alone. In certain embodiments the dose of a co-administered anti-inflammatory agent is lower than the dose that would be administered if the anti-inflammatory agent was administered alone. In certain embodiments the dose of a co-administered anti-inflammatory agent is greater than the dose that would be administered if the anti-inflammatory agent was administered alone.

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

In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition described herein include anti-hyperproliferative agents. In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition described herein include, but are not limited to all-trans retinoic acid, azacitidine, azathioprine, bleomycin, carboplatin, capecitabine, cisplatin, chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabine, hydroxyurea, idarubicin, imatinib, mechlorethamine, mercaptopurine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, valrubicin, vinblastine, vincristine, vindesine, or vinorelbine. In certain embodiments, the anti-hyperproliferative agent is administered prior to administration of a pharmaceutical composition described herein. In certain embodiments, the anti-hyperproliferative agent is administered following administration of a pharmaceutical composition described herein. In certain embodiments the anti-hyperproliferative agent is administered at the same time as a pharmaceutical composition described herein. In certain embodiments the

dose of a co-administered anti-hyperproliferative agent is the same as the dose that would be administered if the anti-hyperproliferative agent was administered alone. In certain embodiments the dose of a co-administered anti-hyperproliferative agent is lower than the dose that would be administered if the anti-hyperproliferative agent was administered alone. In certain embodiments the dose of a co-administered anti-hyperproliferative agent is greater than the dose that would be administered if the anti-hyperproliferative agent was administered alone.

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

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

Certain Comparator Compositions

In certain embodiments, ISIS 407935, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') ATGCATGGTGATGCTTCTGA (incorporated herein as SEQ ID NO: 120), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl moiety, which was previously described in WO 2009/061851, incorporated herein by reference, is a comparator compound.

In certain embodiments, ISIS 407936, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') GGCATTGCGCCACCATGCATG (incorporated herein as SEQ ID NO: 122), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl moiety, which was previously described in WO 2009/061851, incorporated herein by reference, is a comparator compound.

In certain embodiments, ISIS 407939, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') TGCAGCCCGGCACCCAGCGA (incorporated herein as SEQ ID NO: 72), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and

16-20 comprise a 2'-O-methoxyethyl moiety, which was previously described in WO 2009/061851, incorporated herein by reference, is a comparator compound.

In certain embodiments, compounds described herein are more efficacious, potent, and/or tolerable in various in vitro and in vivo systems than ISIS 407935, ISIS 407936, and/or ISIS 407939. ISIS 407935, ISIS 407936, and ISIS 407939 were selected as a comparator compounds because they exhibited high levels of dose-dependent inhibition in various studies as described in WO 2009/061851. Thus, ISIS 407935, ISIS 407936, and ISIS 407939 were deemed highly efficacious and potent compounds. In certain embodiments, other compounds described in WO 2009/061851 are used as comparator compounds.

Certain Compositions

In certain embodiments, ISIS 473589 is more efficacious, potent, and/or tolerable than comparator compositions, such as ISIS 407935, 407936, and/or ISIS 407939.

For example, as provided in Example 1 (hereinbelow), ISIS 473589 achieved 97% inhibition in cultured Hep3B cells when transfected using electroporation with 2,000nM antisense oligonucleotide, whereas ISIS 407939 achieved 80% inhibition. Thus, ISIS 473589 is more efficacious than the comparator compound, ISIS 407939.

In another example, as provided in Example 13 (hereinbelow), ISIS 473589 achieved an IC₅₀ of 0.3 μM in a 5 point dose response curve (0.074 μM, 0.222 μM, 0.667 μM, 2.000 μM, and 6.000 μM) in cultured Hep3B cells when transfected using electroporation, whereas ISIS 407939 achieved an IC₅₀ of 0.9 μM.

Thus, ISIS 473589 is more potent than the comparator compound, ISIS 407939.

In another example, as provided in Example 17 (hereinbelow), ISIS 473589 achieved 96% inhibition when administered subcutaneously twice a week for 3 weeks with 10 mg/kg/week to transgenic mice harboring a Factor VII genomic DNA fragment, whereas ISIS 407935 achieved 80% inhibition. Thus, ISIS 473589 is more efficacious than the comparator compound, ISIS 407939.

In another example, as provided in Example 34 (hereinbelow), ISIS 473589 exhibited more favorable tolerability markers than ISIS 407935 when administered to CD-1 mice. ISIS 473589 was administered subcutaneously twice a week for 6 weeks at 25mg/kg. ISIS 407935 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. After treatment, ALT, AST, and BUN levels were lower in ISIS 473589 treated mice than in ISIS 407935 treated mice. Therefore, ISIS 473589 is more tolerable than the comparator compound, ISIS 407935 in CD-1 mice.

In another example, as provided in Example 35 (hereinbelow), ISIS 473589 exhibited more favorable tolerability markers than ISIS 407935 when administered to Sprague-Dawley rats. ISIS 473589 was administered subcutaneously twice a week for 6 weeks at 25mg/kg. ISIS 407935 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. After treatment, ALT, AST, and BUN levels were lower in ISIS 473589 treated rats than in ISIS 407935 treated rats. Therefore, ISIS 473589 is more tolerable than the comparator compound, ISIS 407935 in Sprague-Dawley rats.

In another example, as provided in Example 38 (hereinbelow), ISIS 473589 achieved 25%, 44%, 62%, and 80% mRNA inhibition and 0%, 6%, 40%, and 78% protein inhibition when administered to transgenic mice harboring a Factor VII genomic DNA fragment subcutaneously twice a week for 3 weeks at 0.625, 1.25, 2.50, and 5.00 mg/kg/week. ISIS 407935 achieved 28%, 45%, 57%, and 85% mRNA inhibition and 3%, 0%, 47%, and 65% protein inhibition when administered to transgenic mice harboring a Factor VII genomic DNA fragment subcutaneously twice a week for 3 weeks at 2.5, 5.0, 10.0, and 20.00 mg/kg/week. Therefore, ISIS 473589 is more efficacious than ISIS 407935.

In another example, as provided in Example 39 (hereinbelow), ISIS 473589 exhibited more favorable tolerability markers in cynomolgous monkeys including complement C3 measurements, kidney function, body and organ weight, and macroscopic observation upon necropsy. Treatment with ISIS 407935 resulted in reduced complement C3 levels, indicating treatment with ISIS 407935 may have resulted in repeated complement activation to a greater degree than ISIS 473589. Treatment with ISIS 407935 resulted in elevated urine protein to creatinine ratio in the monkeys, indicating treatment with ISIS 407935 perturbed kidney function, whereas treatment with 473589 did not have any effect on the kidney function outside the expected range. Treatment with ISIS 407935 resulted in a 2.2-fold increase in spleen weight, a 2.7-fold increase in liver weight, and a 1.3-fold increase in kidney weight compared to the control, indicating that ISIS 407935 had an effect on organ weights, which was not observed with ISIS 473589. ISIS 407935 was observed to result in ascites in 2 out of 4 monkeys suggesting it is less well tolerated than ISIS 473589. Therefore, ISIS 473589 is more tolerable than the comparator compound, ISIS 407935.

In certain embodiments, ISIS 490279 is more efficacious, potent, and/or tolerable than comparator compositions, such as ISIS 407935, 407936, and/or ISIS 407939.

For example, as provided in Example 29 (hereinbelow), ISIS 490279 achieved 59% inhibition when administered subcutaneously twice a week for 3 weeks with 1 mg/kg/week to transgenic mice harboring a Factor VII genomic DNA fragment, whereas ISIS 407936 achieved 28% inhibition. Thus, ISIS 490279 is more efficacious than the comparator compound, ISIS 407936.

In another example, as provided in Example 34 (hereinbelow), ISIS 490279 exhibited more favorable tolerability markers than ISIS 407935 when administered to CD-1 mice. ISIS 490279 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. ISIS 407935 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. After treatment, ALT, AST, and BUN levels were lower in ISIS 490279 treated mice than in ISIS 407935 treated mice. Therefore, ISIS 490279 is more tolerable than the comparator compound, ISIS 407935 in CD-1 mice.

In another example, as provided in Example 35 (hereinbelow), ISIS 490279 was as tolerable or more tolerable than ISIS 407935 when administered to Sprague-Dawley rats. ISIS 490279 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. ISIS 407935 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. After treatment, ALT was lower in ISIS 490279 treated rats than in ISIS

407935 treated rats. Therefore, ISIS 490279 is as tolerable or more tolerable than the comparator compound, ISIS 407935 in Sprague-Dawley rats.

In another example, as provided in Example 38 (hereinbelow), ISIS 490279 achieved 33%, 51%, 70%, and 88% mRNA inhibition and 23%, 31%, 75%, and 91% protein inhibition when administered to transgenic mice harboring a Factor VII genomic DNA fragment subcutaneously twice a week for 3 weeks at 2.5, 5.0, 10.0, and 20.00 mg/kg/week. ISIS 407935 achieved 28%, 45%, 57%, and 85% mRNA inhibition and 3%, 0%, 47%, and 65% protein inhibition when administered to transgenic mice harboring a Factor VII genomic DNA fragment subcutaneously twice a week for 3 weeks at 2.5, 5.0, 10.0, and 20.00 mg/kg/week. Therefore, ISIS 473589 is more efficacious than ISIS 407935.

In another example, as provided in Example 39 (hereinbelow), ISIS 490279 exhibited more favorable tolerability markers in cynomolgous monkeys including complement C3 measurements, kidney function, body and organ weight, and macroscopic observation upon necropsy. Treatment with ISIS 407935 resulted in reduced complement C3 levels, indicating treatment with ISIS 407935 may have resulted in repeated complement activation to a greater degree than ISIS 490279. Treatment with ISIS 407935 resulted in elevated urine protein to creatinine ratio in the monkeys, indicating treatment with ISIS 407935 perturbed kidney function, whereas treatment with 490279 did not have any effect on the kidney function outside the expected range. Treatment with ISIS 407935 resulted in a 2.2-fold increase in spleen weight, a 2.7-fold increase in liver weight, and a 1.3-fold increase in kidney weight compared to the control, indicating that ISIS 407935 had an effect on organ weights, which was not observed with ISIS 490279. ISIS 407935 was observed to result in ascites in 2 out of 4 monkeys suggesting it is less well tolerated than ISIS 490279. Therefore, ISIS 490279 is more tolerable than the comparator compound, ISIS 407935.

In certain embodiments, ISIS 540175 is more efficacious, potent, and/or tolerable than comparator compositions, such as ISIS 407935.

For example, as provided in Example 31 (hereinbelow), ISIS 540175 achieved 55% and 90% inhibition when administered subcutaneously with 0.1 mg/kg/week and 0.3 mg/kg/week to transgenic mice harboring a Factor VII genomic DNA fragment, whereas ISIS 407935 achieved 31% and 65% inhibition when administered at 0.5 mg/kg/week and 1.5 mg/kg/week. Thus, ISIS 540175 is more potent than the comparator compounds, ISIS 407935.

In another example, as provided in Example 34 (hereinbelow), ISIS 540175 exhibited more favorable tolerability markers than ISIS 407935 when administered to CD-1 mice. ISIS 540175 was administered subcutaneously twice a week for 6 weeks at 25mg/kg. ISIS 407935 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. After treatment, ALT and AST levels were lower in ISIS 540175 treated mice than in ISIS 407935 treated mice. Therefore, ISIS 540175 is more tolerable than the comparator compound, ISIS 407935 in CD-1 mice.

In another example, as provided in Example 35 (hereinbelow), ISIS 540175 exhibited more favorable tolerability markers than ISIS 407935 when administered to Sprague-Dawley rats. ISIS 540175 was

administered subcutaneously twice a week for 6 weeks at 25mg/kg. ISIS 407935 was administered subcutaneously twice a week for 6 weeks at 50mg/kg. After treatment, ALT, AST, and BUN levels were lower in ISIS 540175 treated rats than in ISIS 407935 treated rats. Therefore, ISIS 540175 is more tolerable than the comparator compound, ISIS 407935 in Sprague-Dawley rats.

5 In another example, as provided in Example 38 (hereinbelow), ISIS 540175 achieved 55%, 65%, 85%, and 95% mRNA inhibition and 24%, 49%, 83%, and 93% protein inhibition when administered to transgenic mice harboring a Factor VII genomic DNA fragment subcutaneously twice a week for 3 weeks at 0.625, 1.25, 2.50, and 5.00 mg/kg/week. ISIS 407935 achieved 28%, 45%, 57%, and 85% mRNA inhibition and 3%, 0%, 47%, and 65% protein inhibition when administered to transgenic mice harboring a Factor VII
10 genomic DNA fragment subcutaneously twice a week for 3 weeks at 2.5, 5.0, 10.0, and 20.00 mg/kg/week. Therefore, ISIS 540175 is more efficacious than ISIS 407935.

In another example, as provided in Example 39 (hereinbelow), ISIS 540175 exhibited more favorable tolerability markers in cynomolgous monkeys including complement C3 measurements, kidney function, body and organ weight, and macroscopic observation upon necropsy. Treatment with ISIS 540175 resulted
15 in reduced complement C3 levels, indicating treatment with ISIS 407935 may have resulted in repeated complement activation to a greater degree than ISIS 540175. Treatment with ISIS 407935 resulted in elevated urine protein to creatinine ratio in the monkeys, indicating treatment with ISIS 407935 perturbed kidney function, whereas treatment with 540175 did not have any effect on the kidney function outside the expected range. Treatment with ISIS 407935 resulted in a 2.2-fold increase in spleen weight, a 2.7-fold increase in
20 liver weight, and a 1.3-fold increase in kidney weight compared to the control, indicating that ISIS 407935 had an effect on organ weights, which was not observed with ISIS 540175. ISIS 407935 was observed to result in ascites in 2 out of 4 monkeys suggesting it is less well tolerated than ISIS 540175. Therefore, ISIS 540175 is more tolerable than the comparator compound, ISIS 407935.

In another example, as provided in Example 40 (hereinbelow), ISIS 540175 achieved an IC_{50} of 0.2
25 μ M in a 5 point dose response curve (0.003 μ M, 0.016 μ M, 0.800 μ M, 4.000 μ M, and 20.000 μ M) in cultured HepG2 cells when transfected using electroporation, whereas ISIS 407935 achieved an IC_{50} of 0.4 μ M. Thus, ISIS 540175 is more potent than the comparator compound, ISIS 407935.

EXAMPLES

30 *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: Modified antisense oligonucleotides comprising cEt and MOE modifications targeting human coagulation Factor VII

Antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. ISIS 407939 (described hereinabove), which was described in an earlier publication (WO 2009/061851) was also tested.

The newly designed modified antisense oligonucleotides and their motifs are described in Table 1. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The 'Sugar Chemistry' column provides the sugar modifications throughout each oligonucleotide: 'd' indicates a 2'-deoxynucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'e' indicates a 2'-O-methoxyethyl nucleoside. The 'Sequence' column provides the nucleobase sequence for each SEQ ID NO.

Each oligonucleotide listed in Table 1 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence. Oligonucleotides having multiple start and stop sites target a region that is repeated within a Factor VII sequence (e.g., within SEQ ID NO: 1).

Activity of the newly designed oligonucleotides was compared to ISIS 407939. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (forward sequence GGGACCCTGATCAACACCAT, designated herein as SEQ ID NO: 5; reverse sequence CCAGTTCTTGATTTTGTGCGAAACA, designated herein as SEQ ID NO: 6; probe sequence TGGGTGGTCTCCGCGGCC, designated herein as SEQ ID NO: 7) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 771 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Table 1. Each of the newly designed antisense oligonucleotides provided in Table 1 achieved greater than 80% inhibition and, therefore, are more active than ISIS 407939.

Table 1

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to
SEQ ID NO: 1 and SEQ ID NO: 2

SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	Sequence	SEQ ID NO	ISIS NO	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sugar Chemistry
15255	15274	TGCAGCCCGGCACCCAGCGA	72	407939	80	2312	2331	eeeeeddddddddeeeeee
1147	1162	GATGAAATCTCTGCAG	21	473359	92	36	51	kkkdddskddddddee
1154	1169	AGACCATGATGAAATC	22	473360	96	43	58	kkkdddskddddddee
1382	1397	GCCTGGATGCTGGTTT	23	473168	94	n/a	n/a	kkkdddddddddskkk
1382	1397	GCCTGGATGCTGGTTT	23	473317	95	n/a	n/a	kkkddddddddddee
1382	1397	GCCTGGATGCTGGTTT	23	473471	90	n/a	n/a	kkkdddskddddddee
1382	1397	GCCTGGATGCTGGTTT	23	473620	94	n/a	n/a	kdkdkddddddddddee
1383	1396	CCTGGATGCTGGTT	24	473019	88	n/a	n/a	kkdddddddddskk
1384	1397	GCCTGGATGCTGGT	25	473020	93	n/a	n/a	kkdddddddddskk
2369	2384	TGGAGCGGTCACCTCC	26	473321	93	n/a	n/a	kkkddddddddddee
4717	4732	AGGAGGCTGAGGATGC	27	473322	94	n/a	n/a	kkkddddddddddee
4871	4886	CTGCAGGAGCGGCCTA	28	473323	96	n/a	n/a	kkkddddddddddee
6411	6426	CGTATTTTCTGATGTG	29	473326	94	n/a	n/a	kkkddddddddddee
6411	6426	CGTATTTTCTGATGTG	29	473480	92	n/a	n/a	kkkdddskddddddee
6642	6657	GAGGTGACCCGTGAGC	30	473178	96	n/a	n/a	kkkdddddddddskkk
6642	6657	GAGGTGACCCGTGAGC	30	473327	96	n/a	n/a	kkkddddddddddee
6642	6657	GAGGTGACCCGTGAGC	30	473481	93	n/a	n/a	kkkdddskddddddee
6642	6657	GAGGTGACCCGTGAGC	30	473630	89	n/a	n/a	kdkdkddddddddddee
6643	6656	AGGTGACCCGTGAG	31	473029	96	n/a	n/a	kkdddddddddskk
6765	6778							
6887	6900							
6953	6966							
7071	7084							
7189	7202							
7243	7256							
11017	11030	CTGCTCACAGCCGC	32	472925	93	452	465	kkdddddddddskk
11023	11036	GCAGTACTGCTCAC	33	472926	85	458	471	kkdddddddddskk
11839	11854	AATGGTCAGGGCTGGT	34	473195	97	n/a	n/a	kkkdddddddddskkk
11840	11853	ATGGTCAGGGCTGG	35	473046	90	n/a	n/a	kkdddddddddskk
12128	12141	GGTTTGCTGGCATT	36	472935	92	598	611	kkdddddddddskk
12141	12156	ACAATTCGGCCTTGGG	37	473089	95	611	626	kkkdddddddddskkk
12629	12644	GCTCAGACCTGGCTCT	38	473350	93	n/a	n/a	kkkddddddddddee
12633	12648	AGCTGCTCAGACCTGG	39	473353	93	n/a	n/a	kkkddddddddddee
12634	12647	GCTGCTCAGACCTG	40	473055	91	n/a	n/a	kkdddddddddskk
12842	12857	CCACCCAGATGGTGTT	41	473392	95	715	730	kkkdddskddddddee

12863	12878	CGAAACAGTGGGCCGC	42	473095	100	736	751	kkkddddddddddkkk
12863	12878	CGAAACAGTGGGCCGC	42	473244	99	736	751	kkkdddddddddeee
12863	12878	CGAAACAGTGGGCCGC	42	473393	99	736	751	kkkdddskddddddee
12863	12878	CGAAACAGTGGGCCGC	42	473547	98	736	751	kdkdkdddddddee
12864	12877	GAAACAGTGGGCCG	43	472942	87	737	750	kkdddddddddkk
13741	13756	GTGCTCGCTGAGGTCG	44	473098	97	798	813	kkkddddddddkkk
13988	14003	CCATGAGCTCCAGGGC	45	473408	92	1045	1060	kkkdddskddddddee
14019	14032	CTGGGTCATCAGCC	46	472958	89	1076	1089	kkdddddddddkk
14022	14035	GTCCTGGGTCATCA	47	472959	90	1079	1092	kkdddddddddkk
14079	14094	CAGAACATGTACTCCG	48	473566	94	1136	1151	kdkdkdddddddee
14092	14107	CGAGTAGCCGGCACAG	49	473567	95	1149	1164	kdkdkdddddddee
14128	14143	TCCACTGTCCCCCTTG	50	473569	92	1185	1200	kdkdkdddddddee
14232	14245	CCTGGTGTACACCC	51	457851	90	1289	1302	kkdddddddddkk
14244	14257	GTAAGTGGGAGACCC	32	472970	91	1301	1314	kkdddddddddkk
14612	14627	CCCCTCTGTCCAGCGC	53	473125	90	1669	1684	kkkddddddddkkk
14612	14627	CCCCTCTGTCCAGCGC	53	473274	98	1669	1684	kkkdddddddddeee
14612	14627	CCCCTCTGTCCAGCGC	53	473428	90	1669	1684	kkkdddskddddddee
14612	14627	CCCCTCTGTCCAGCGC	53	473577	93	1669	1684	kdkdkdddddddee
14613	14626	CCCTCTGTCCAGCG	54	472976	97	1670	1683	kkdddddddddkk
14709	14722	AGGCCAGCAGATCA	55	472983	94	1766	1779	kkdddddddddkk
14714	14727	GCCTGAGGCCAGCA	56	472984	90	1771	1784	kkdddddddddkk
15097	15112	ATGGAGTCAGCATCGG	57	473135	97	2154	2169	kkkddddddddkkk
15098	15111	TGGAGTCAGCATCG	58	472986	95	2155	2168	kkdddddddddkk
15128	15143	GCTAAACAACCGCCTT	59	473137	95	2185	2200	kkkddddddddkkk
15128	15143	GCTAAACAACCGCCTT	59	473286	95	2185	2200	kkkdddddddddeee
15128	15143	GCTAAACAACCGCCTT	59	473440	88	2185	2200	kkkdddskddddddee
15128	15143	GCTAAACAACCGCCTT	59	473589	97	2185	2200	kdkdkdddddddee
15129	15142	CTAAACAACCGCCT	60	472988	85	2186	2199	kkdddddddddkk
15164	15179	TGAAGATGATAATGGA	61	473140	96	2221	2236	kkkddddddddkkk
15165	15178	GAAGATGATAATGG	62	472991	90	2222	2235	kkdddddddddkk
15181	15196	TTCTGAATTGTCTGAA	63	473444	94	2238	2253	kkkdddskddddddee
15188	15203	GTGATGCTTCTGAATT	64	473142	96	2245	2260	kkkddddddddkkk
15188	15203	GTGATGCTTCTGAATT	64	473291	95	2245	2260	kkkdddddddddeee
15188	15203	GTGATGCTTCTGAATT	64	473594	95	2245	2260	kdkdkdddddddee
15190	15205	TGGTGATGCTTCTGAA	65	473143	97	2247	2262	kkkddddddddkkk
15190	15205	TGGTGATGCTTCTGAA	65	473292	96	2247	2262	kkkdddddddddeee
15190	15205	TGGTGATGCTTCTGAA	65	473446	96	2247	2262	kkkdddskddddddee
15190	15205	TGGTGATGCTTCTGAA	65	473595	84	2247	2262	kdkdkdddddddee
15191	15204	GGTGATGCTTCTGA	66	472994	96	2248	2261	kkdddddddddkk
15191	15206	ATGGTGATGCTTCTGA	67	473144	98	2248	2263	kkkddddddddkkk
15191	15206	ATGGTGATGCTTCTGA	67	473293	96	2248	2263	kkkdddddddddeee
15192	15205	TGGTGATGCTTCTG	68	472995	96	2249	2262	kkdddddddddkk
15194	15209	TGCATGGTGATGCTTC	69	473294	91	2251	2266	kkkdddddddddeee

15194	15209	TGCATGGTGATGCTTC	69	473597	94	2251	2266	kdkdkdddddddee
15195	15208	GCATGGTGATGCTT	70	472996	94	2252	2265	kkdddddddddkk
15195	15210	ATGCATGGTGATGCTT	71	473295	92	2252	2267	kkkddddddddddee
15262	15277	CTGTGCAGCCCGGCAC	73	473296	98	2319	2334	kkkddddddddddee
15262	15277	CTGTGCAGCCCGGCAC	73	473450	95	2319	2334	kkkdddskddddddee
15263	15276	TGTGCAGCCCGGCA	74	472998	97	2320	2333	kkdddddddddskk

Example 2: Modified antisense oligonucleotides comprising cEt, MOE, and 3'-fluoro-HNA modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. ISIS 407939 was also tested.

The newly designed modified antisense oligonucleotides and their motifs are described in Table 2. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The 'Sugar Chemistry' column provides the sugar modifications throughout each oligonucleotide: 'd' indicates a 2'-deoxynucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, 'e' indicates a 2'-O-methoxyethyl nucleoside, and 'g' indicates a 3'-fluoro-HNA nucleoside. The 'Sequence' column provides the nucleobase sequence for each SEQ ID NO..

Each oligonucleotide listed in Table 2 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence. Oligonucleotides having multiple start and stop sites target a region that is repeated within a Factor VII sequence (e.g., within SEQ ID NO: 1).

Activity of the newly designed oligonucleotides was compared to ISIS 407939. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 765 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Table 2. All but one of the newly designed antisense oligonucleotides provided in Table 2 achieved greater than 30% inhibition and, therefore, are more active than ISIS 407939.

Table 2

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to
SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	% inhibition	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Sugar Chemistry
15255	15274	TGCAGCCCGGCACCCA GCGA	72	407939	30	2312	2331	eeeeeddddddddeeeee
1384	1397	GCCTGGATGCTGGT	25	482838	81	n/a	n/a	ggdddddddddgg
4871	4886	CTGCAGGAGCGGCCTA	28	482992	93	n/a	n/a	gggdddddddddggg
6642	6657	GAGGTGACCCGTGAGC	30	482996	97	n/a	n/a	gggdddddddddggg
1382	1397	GCCTGGATGCTGGTTT	23	483284	82	n/a	n/a	gdgdgdddddddeee
4717	4732	AGGAGGCTGAGGATGC	27	483289	70	n/a	n/a	gdgdgdddddddeee
4871	4886	CTGCAGGAGCGGCCTA	28	483290	80	n/a	n/a	gdgdgdddddddeee
6642	6657	GAGGTGACCCGTGAGC	30	483294	69	n/a	n/a	gdgdgdddddddeee
1382	1397	GCCTGGATGCTGGTTT	23	483438	81	n/a	n/a	ggddddddddddeeee
4871	4886	CTGCAGGAGCGGCCTA	28	483444	84	n/a	n/a	ggddddddddddeeee
6642	6657	GAGGTGACCCGTGAGC	30	483448	77	n/a	n/a	ggddddddddddeeee
6643	6656	AGGTGACCCGTGAG	31	482847	79	n/a	n/a	ggdddddddddgg
6765	6778							
6887	6900							
6953	6966							
7071	7084							
7189	7202							
7243	7256							
11017	11030	CTGCTCACAGCCGC	32	482747	85	452	465	ggdddddddddgg
12634	12647	GCTGCTCAGACCTG	40	482873	81	n/a	n/a	ggdddddddddgg
12635	12648	AGCTGCTCAGACCT	75	482874	82	n/a	n/a	ggdddddddddgg
12636	12649	AAGCTGCTCAGACC	76	482875	82	n/a	n/a	ggdddddddddgg
11016	11031	ACTGCTCACAGCCGCC	77	482896	95	451	466	gggdddddddddggg
12629	12644	GCTCAGACCTGGCTCT	38	483019	89	n/a	n/a	gggdddddddddggg
11016	11031	ACTGCTCACAGCCGCC	77	483045	92	451	466	gdgdddddddddgdg
11016	11031	ACTGCTCACAGCCGCC	77	483194	64	451	466	gdgdgdddddddeee
12629	12644	GCTCAGACCTGGCTCT	38	483317	79	n/a	n/a	gdgdgdddddddeee
11016	11031	ACTGCTCACAGCCGCC	57	483343	75	451	466	ggddddddddddeeee
12629	12644	GCTCAGACCTGGCTCT	38	483471	76	n/a	n/a	ggddddddddddeeee
12941	12956	ACCCAGCACCGCGGTC	78	483478	20	n/a	n/a	ggddddddddddeeee
12978	12993							
13015	13030							
13052	13067							
13089	13104							

14093	14106	GAGTAGCCGGCACA	79	482784	83	1150	1163	ggdddddggg
14613	14626	CCCTCTGTCCAGCG	54	482794	91	1670	1683	ggdddddggg
15098	15111	TGGAGTCAGCATCG	58	482804	80	2155	2168	ggdddddggg
15191	15204	GGTGATGCTTCTGA	66	482812	81	2248	2261	ggdddddggg
15192	15205	TGGTGATGCTTCTG	68	482813	92	2249	2262	ggdddddggg
15195	15208	GCATGGTGATGCTT	70	482814	94	2252	2265	ggdddddggg
15196	15209	TGCATGGTGATGCT	80	482815	81	2253	2266	ggdddddggg
15263	15276	TGTGCAGCCCGGCA	74	482816	71	2320	2333	ggdddddggg
13741	13756	GTGCTCGCTGAGGTCG	44	482916	90	798	813	gggdddddggg
14079	14094	CAGAACATGTACTCCG	48	482932	89	1136	1151	gggdddddggg
15097	15112	ATGGAGTCAGCATCGG	57	482953	93	2154	2169	gggdddddggg
15191	15206	ATGGTGATGCTTCTGA	67	482962	97	2248	2263	gggdddddggg
15194	15209	TGCATGGTGATGCTTC	69	482963	96	2251	2266	gggdddddggg
15262	15277	CTGTGCAGCCCGGCAC	73	482965	89	2319	2334	gggdddddggg
13741	13756	GTGCTCGCTGAGGTCG	44	483065	69	798	813	gdgdddddgdg
14612	14627	CCCCTCTGTCCAGCGC	53	483092	89	1669	1684	gdgdddddgdg
14612	14627	CCCCTCTGTCCAGCGC	53	483241	79	1669	1684	gdgdgddddddee
15128	15143	GCTAAACAACCGCCTT	59	483253	76	2185	2200	gdgdgddddddee
15188	15203	GTGATGCTTCTGAATT	64	483258	70	2245	2260	gdgdgddddddee
15191	15206	ATGGTGATGCTTCTGA	67	483260	62	2248	2263	gdgdgddddddee
15194	15209	TGCATGGTGATGCTTC	69	483261	76	2251	2266	gdgdgddddddee
15195	15210	ATGCATGGTGATGCTT	71	483262	75	2252	2267	gdgdgddddddee
15262	15277	CTGTGCAGCCCGGCAC	73	483263	73	2319	2334	gdgdgddddddee
13760	13775	GGCTCTGCTCATCCCC	81	483364	78	817	832	ggddddddeeee
14612	14627	CCCCTCTGTCCAGCGC	53	483395	86	1669	1684	ggddddddeeee
15190	15205	TGGTGATGCTTCTGAA	65	483413	83	2247	2262	ggddddddeeee
15191	15206	ATGGTGATGCTTCTGA	67	483414	76	2248	2263	ggddddddeeee
15194	15209	TGCATGGTGATGCTTC	69	483415	85	2251	2266	ggddddddeeee
15195	15210	ATGCATGGTGATGCTT	71	483416	77	2252	2267	ggddddddeeee
15262	15277	CTGTGCAGCCCGGCAC	73	483417	83	2319	2334	ggddddddeeee

Example 3: Modified oligonucleotides comprising MOE, and/or cEt modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. Also tested were ISIS 403052, ISIS 407594, ISIS 407606, ISIS 407939, and ISIS 416438, which are 5-10-5 MOE gapmers described in an earlier publication (WO 2009/061851).

The newly designed modified antisense oligonucleotides and their motifs are described in Table 3. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in

the oligonucleotides are 5-methylcytosines. The 'Sugar Chemistry' column provides the sugar modifications throughout each oligonucleotide: 'd' indicates a 2'-deoxynucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'e' indicates a 2'-O-methoxyethyl nucleoside. The 'Sequence' column provides the nucleobase sequence for each SEQ ID NO.

5 Each oligonucleotide listed in Table 3 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Activity of the newly designed gapmers was compared to ISIS 403052, ISIS 407594, ISIS 407606, ISIS 407939, and ISIS 416438. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 380 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Table 3. Each of the newly designed antisense oligonucleotides provided in Table 3 achieved greater than 64% inhibition and, therefore, are more active than each of ISIS 403052, ISIS 407594, ISIS 407606, ISIS 407939, and ISIS 416438.

Table 3

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	% inhibition	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Sugar Chemistry
n/a	n/a	GGCACACTGGTCCCCATCAC	82	403052	64	299	318	eeeeeddddddddeeeee
1208	1227	CCTGCAGCCAGGCAGCCCTG	83	407594	40	n/a	n/a	eeeeeddddddddeeeee
9204	9223	CTGGTCCTTGCAGGAGCCCC	84	407606	39	338	357	eeeeeddddddddeeeee
15255	15274	TGCAGCCCGGCACCCAGCGA	72	407939	57	2312	2331	eeeeeddddddddeeeee
9194	9213	CAGGAGCCCCCATTTCTGGCA	85	416438	62	328	347	eeeeeddddddddeeeee
11016	11031	ACTGCTCACAGCCGCC	77	484487	91	451	466	kdkdddddddddskdk
14612	14627	CCCCTCTGTCCAGCGC	53	484539	92	1669	1684	kdkdddddddddskdk
14708	14723	GAGGCCAGCAGATCAC	86	484546	92	1765	1780	kdkdddddddddskdk
14713	14728	AGCCTGAGGCCAGCAG	87	484547	89	1770	1785	kdkdddddddddskdk
15097	15112	ATGGAGTCAGCATCGG	57	484549	91	2154	2169	kdkdddddddddskdk
15190	15205	TGGTGATGCTTCTGAA	65	484557	92	2247	2262	kdkdddddddddskdk

15191	15206	ATGGTGATGCTTCTGA	67	484558	94	2248	2263	kdkskkkkkkkkkkkk
15194	15209	TGCATGGTGATGCTTC	69	484559	90	2251	2266	kdkskkkkkkkkkkkk
1382	1397	GCCTGGATGCTGGTTT	23	484582	88	n/a	n/a	kdkskkkkkkkkkkkk
9170	9185	AGGCACACTGGTCCCC	88	484632	90	304	319	kkkkkkkkkkkkdeeee
11016	11031	ACTGCTCACAGCCGCC	77	484641	91	451	466	kkkkkkkkkkkkdeeee
14092	14107	CGAGTAGCCGGCACAG	49	484679	90	1149	1164	kkkkkkkkkkkkdeeee
14612	14627	CCCCTCTGTCCAGCGC	53	484693	93	1669	1684	kkkkkkkkkkkkdeeee
15190	15205	TGGTGATGCTTCTGAA	65	484711	92	2247	2262	kkkkkkkkkkkkdeeee
15191	15206	ATGGTGATGCTTCTGA	67	484712	92	2248	2263	kkkkkkkkkkkkdeeee
15194	15209	TGCATGGTGATGCTTC	69	484713	85	2251	2266	kkkkkkkkkkkkdeeee
15195	15210	ATGCATGGTGATGCTT	71	484714	83	2252	2267	kkkkkkkkkkkkdeeee
15262	15277	CTGTGCAGCCCGGCAC	73	484715	93	2319	2334	kkkkkkkkkkkkdeeee
1382	1397	GCCTGGATGCTGGTTT	23	484736	89	n/a	n/a	kkkkkkkkkkkkdeeee
4871	4886	CTGCAGGAGCGGCCTA	28	484742	93	n/a	n/a	kkkkkkkkkkkkdeeee
6642	6657	GAGGTGACCCGTGAGC	30	484746	88	n/a	n/a	kkkkkkkkkkkkdeeee
12631	12646	CTGCTCAGACCTGGCT	89	484771	89	n/a	n/a	kkkkkkkkkkkkdeeee

Example 4: Modified antisense oligonucleotides comprising MOE modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. Also tested were ISIS 403094, ISIS 407641, ISIS 407643, ISIS 407662, ISIS 407900, ISIS 407910, ISIS 407935, ISIS 407936, ISIS 407939, ISIS 416446, ISIS 416449, ISIS 416455, ISIS 416472, ISIS 416477, ISIS 416507, ISIS 416508, ISIS 422086, ISIS 422087, ISIS 422140, and ISIS 422142, which are 5-10-5 MOE gapmers targeting human Factor VII and are described in an earlier publication (WO 2009/061851).

The newly designed modified antisense oligonucleotides in Table 4 were designed as 5-10-5 MOE gapmers. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosine residues throughout each oligonucleotide are 5-methylcytosines.

Each oligonucleotide listed in Table 4 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. Each oligonucleotide listed in Table 5 is targeted to human Factor VII gene sequence DB184141.1, designated herein as SEQ ID NO: 3. “Start site” indicates the 5’-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. “Stop site”

indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Activity of the newly designed oligonucleotides was compared to ISIS 403094, ISIS 407641, ISIS 407643, ISIS 407662, ISIS 407900, ISIS 407910, ISIS 407935, ISIS 407936, ISIS 407939, ISIS 416446, ISIS 416449, ISIS 416455, ISIS 416472, ISIS 416477, ISIS 416507, ISIS 416508, ISIS 422086, ISIS 422087, ISIS 422140, and ISIS 422142. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 916 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Tables 4 and 5.

Table 4

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	ISIS No	% inhibition	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	SEQ ID NO
14240	14259	ATGTACTGGGAGACCCTGGT	403094	60	1297	1316	110
14707	14726	CCTGAGGCCAGCAGATCACG	407641	64	1764	1783	112
15098	15117	CACACATGGAGTCAGCATCG	407643	78	2155	2174	114
12141	12160	CCCCACAATTCGGCCTTGGG	407900	66	611	630	105
14104	14123	AGTCCTTGCTGCCATCCGAG	407910	25	1161	1180	108
15191	15210	ATGCATGGTGATGCTTCTGA	407935	91	2248	2267	120
15204	15223	GGCATTCGCCACCATGCATG	407936	80	2261	2280	122
15255	15274	TGCAGCCCGGCACCCAGCGA	407939	67	2312	2331	72
11024	11043	GGTCACTGCAGTACTGCTCA	416446	73	459	478	103
12094	12113	TAGGTATTTTCCACATGGA	416449	33	564	583	104
13760	13779	CGCCGGCTCTGCTCATCCCC	416455	42	817	836	107
14348	14367	CAGCCTTGGCTTTCTCTCCA	416472	78	1405	1424	111
14710	14729	CAGCCTGAGGCCAGCAGATC	416477	25	1767	1786	113
4847	4866	GGTACTGAGCGCGAAGAA	416507	73	n/a	n/a	97
4873	4892	CGAGTTCTGCAGGAGCGGCC	416508	75	n/a	n/a	100
15190	15209	TGCATGGTGATGCTTCTGAA	422086	90	2247	2266	119
15192	15211	CATGCATGGTGATGCTTCTG	422087	89	2249	2268	121
4870	4889	GTTCTGCAGGAGCGGCCTAA	422140	59	n/a	n/a	98
4872	4891	GAGTTCTGCAGGAGCGGCCT	422142	73	n/a	n/a	99
1383	1402	CCTGTGCCTGGATGCTGGTT	490275	35	n/a	n/a	90
1385	1404	CTCCTGTGCCTGGATGCTGG	490277	73	n/a	n/a	91
1386	1405	CCTCCTGTGCCTGGATGCTG	490278	78	n/a	n/a	92

1387	1406	CCCTCCTGTGCCTGGATGCT	490279	66	n/a	n/a	93
2228	2247	GGCAGTCCCTGCTCACCTCT	490323	65	n/a	n/a	94
2487	2506	GCATCAGAAAAGCTCTCAAG	490368	78	n/a	n/a	95
4725	4744	GTCTGGTTTGAAGGAGGCT	490396	76	n/a	n/a	96
4939	4958	GGAGGGACGACCTTTGCTGG	490424	57	n/a	n/a	101
10676	10695	GACCACTCTTCCGAGCAGCT	490803	70	n/a	n/a	102
12801	12820	CTGAGCTCCATTCACCAACA	490103	87	674	693	106
14232	14251	GGAGACCCTGGTGTACACCC	490149	82	1289	1308	109
15129	15148	TGAGAGCTAAACAACCGCCT	490196	81	2186	2205	115
15130	15149	GTGAGAGCTAAACAACCGCC	490197	85	2187	2206	116
15183	15202	TGATGCTTCTGAATTGTCTG	490208	89	2240	2259	117
15184	15203	GTGATGCTTCTGAATTGTCT	490209	81	2241	2260	118

Table 5

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 3

Start Site on SEQ ID NO: 3	Stop Site on SEQ ID NO: 3	Sequence	ISIS No	% inhibition	SEQ ID NO
50	69	TCCTGCAGCCAGGCAGCCCT	407662	76	123
444	463	GGTCACTGCAGTACTGCTCA	416446	73	103

5

Example 5: Modified antisense oligonucleotides comprising cEt modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. Also tested was ISIS 407939, a 5-10-5 MOE gapmer targeting human Factor VII, which was described in an earlier publication (WO 2009/061851). ISIS 457851, ISIS 472925, ISIS 472926, ISIS 472935, ISIS 472942, ISIS 472958, ISIS 472959, ISIS 472970, ISIS 472976, ISIS 472983, ISIS 472984, ISIS 472988, ISIS 472991, ISIS 472994, ISIS 472995, ISIS 472996, ISIS 472998, and ISIS 473020, described in the Examples above were also included in the screen.

The newly designed modified antisense oligonucleotides in Table 6 were designed as 2-10-2 cEt gapmers. The 2-10-2 cEt gapmers are 14 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising two nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each oligonucleotide are 5-methylcytosines.

Each oligonucleotide listed in Table 6 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which

the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Activity of the newly designed oligonucleotides was compared to ISIS 407939. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 614 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Table 6. Many of the newly designed antisense oligonucleotides provided in Table 6 achieved greater than 72% inhibition and, therefore, are more active than ISIS 407939.

Table 6

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	ISIS No	% inhibition	Motif	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	SEQ ID NO
15255	15274	TGCAGCCCCGGCACCCAGCGA	407939	72	5-10-5	2312	2331	72
1384	1397	GCCTGGATGCTGGT	473020	90	2-10-2	n/a	n/a	25
1386	1399	GTGCCTGGATGCTG	492465	83	2-10-2	n/a	n/a	124
1388	1401	CTGTGCCTGGATGC	492467	74	2-10-2	n/a	n/a	125
2237	2250	AGTGGCAGTCCCTG	492492	84	2-10-2	n/a	n/a	126
2239	2252	CCAGTGGCAGTCCC	492494	91	2-10-2	n/a	n/a	127
2274	2287	GGTGATGTTGGCCC	492503	89	2-10-2	n/a	n/a	128
2482	2495	GCTCTCAAGAACTG	492530	91	2-10-2	n/a	n/a	129
2493	2506	GCATCAGAAAAGCT	492534	91	2-10-2	n/a	n/a	130
2499	2512	AGATTTGCATCAGA	492536	90	2-10-2	n/a	n/a	131
4711	4724	GAGGATGCAGGCGG	492541	84	2-10-2	n/a	n/a	132
4730	4743	TCTGGTTTGGAAGG	492545	89	2-10-2	n/a	n/a	133
4852	4865	GTTACTGAGCGCGG	492566	90	2-10-2	n/a	n/a	134
4874	4887	TCTGCAGGAGCGGC	492571	82	2-10-2	n/a	n/a	135
4875	4888	TTCTGCAGGAGCGG	492572	89	2-10-2	n/a	n/a	136
4876	4889	GTTCTGCAGGAGCG	492573	90	2-10-2	n/a	n/a	137
4877	4890	AGTTCTGCAGGAGC	492574	92	2-10-2	n/a	n/a	138
4878	4891	GAGTTCTGCAGGAG	492575	88	2-10-2	n/a	n/a	139
4923	4936	GGACGAGGCCTCAG	492593	83	2-10-2	n/a	n/a	140
5133	5146	GCTGTGGGCACCAC	492617	91	2-10-2	n/a	n/a	141
5134	5147	AGCTGTGGGCACCA	492618	92	2-10-2	n/a	n/a	142
5135	5148	GAGCTGTGGGCACC	492619	90	2-10-2	n/a	n/a	143

5199	5212	GCTCCGAGCAGGCC	492621	75	2-10-2	n/a	n/a	144
6077	6090	CGGCCGCAGCTCCT	492104	89	2-10-2	182	195	145
6078	6091	CCGGCCGCAGCTCC	492105	86	2-10-2	183	196	146
10988	11001	AGATCAGCTGGTCA	492189	88	2-10-2	423	436	147
11015	11028	GCTCACAGCCGCCG	492194	92	2-10-2	450	463	148
11016	11029	TGCTCACAGCCGCC	492195	90	2-10-2	451	464	149
11017	11030	CTGCTCACAGCCGC	472925	87	2-10-2	452	465	32
11018	11031	ACTGCTCACAGCCG	492196	91	2-10-2	453	466	150
11023	11036	GCAGTACTGCTCAC	472926	88	2-10-2	458	471	33
11030	11043	GGTCACTGCAGTAC	492205	92	2-10-2	465	478	151
12083	12096	GGATATTCAACTGT	492215	77	2-10-2	553	566	152
12099	12112	AGGTATTTTTCCAC	492221	79	2-10-2	569	582	153
12128	12141	GGTTTGCTGGCATT	472935	82	2-10-2	598	611	36
12796	12809	TCACCAACAACAGG	492234	86	2-10-2	669	682	154
12864	12877	GAAACAGTGGGCCG	472942	85	2-10-2	737	750	43
13778	13791	ACCTGCGCCACCCG	492276	75	2-10-2	835	848	155
13779	13792	GACCTGCGCCACCC	492277	75	2-10-2	836	849	156
13893	13906	CCGTTCGGGCAGGC	492306	85	2-10-2	950	963	157
14018	14031	TGGGTCATCAGCCG	492317	93	2-10-2	1075	1088	158
14019	14032	CTGGGTCATCAGCC	472958	92	2-10-2	1076	1089	46
14022	14035	GTCCTGGGTCATCA	472959	88	2-10-2	1079	1092	47
14077	14090	ACATGTACTCCGTG	492329	88	2-10-2	1134	1147	159
14079	14092	GAACATGTACTCCG	492331	95	2-10-2	1136	1149	160
14094	14107	CGAGTAGCCGGCAC	492333	85	2-10-2	1151	1164	161
14095	14108	CCGAGTAGCCGGCA	492334	88	2-10-2	1152	1165	162
14232	14245	CCTGGTGTACACCC	457851	89	2-10-2	1289	1302	51
14244	14257	GTA CTGGGAGACCC	472970	92	2-10-2	1301	1314	52
14265	14278	GAGCTTTTGCAGCC	492365	69	2-10-2	1322	1335	163
14613	14626	CCCTCTGTCCAGCG	472976	94	2-10-2	1670	1683	54
14709	14722	AGGCCAGCAGATCA	472983	76	2-10-2	1766	1779	55
14714	14727	GCCTGAGGCCAGCA	472984	72	2-10-2	1771	1784	56
14741	14754	GTCTCCAGCAATGA	492377	70	2-10-2	1798	1811	164
15101	15114	ACATGGAGTCAGCA	492380	80	2-10-2	2158	2171	165
15105	15118	GCACACATGGAGTC	492384	61	2-10-2	2162	2175	166
15129	15142	CTAAACAACCGCCT	472988	59	2-10-2	2186	2199	60
15130	15143	GCTAAACAACCGCC	492388	70	2-10-2	2187	2200	167
15131	15144	AGCTAAACAACCGC	492389	70	2-10-2	2188	2201	168
15132	15145	GAGCTAAACAACCG	492390	89	2-10-2	2189	2202	169
15133	15146	AGAGCTAAACAACC	492391	80	2-10-2	2190	2203	170
15165	15178	GAAGATGATAATGG	472991	84	2-10-2	2222	2235	62
15184	15197	CTTCTGAATTGTCT	492398	88	2-10-2	2241	2254	171
15185	15198	GCTTCTGAATTGTC	492399	94	2-10-2	2242	2255	172
15187	15200	ATGCTTCTGAATTG	492401	91	2-10-2	2244	2257	173

15190	15203	GTGATGCTTCTGAA	492403	78	2-10-2	2247	2260	174
15191	15204	GGTGATGCTTCTGA	472994	95	2-10-2	2248	2261	66
15192	15205	TGGTGATGCTTCTG	472995	91	2-10-2	2249	2262	68
15193	15206	ATGGTGATGCTTCT	492404	84	2-10-2	2250	2263	175
15194	15207	CATGGTGATGCTTC	492405	87	2-10-2	2251	2264	176
15195	15208	GCATGGTGATGCTT	472996	85	2-10-2	2252	2265	70
15197	15210	ATGCATGGTGATGC	492406	43	2-10-2	2254	2267	177
15263	15276	TGTGCAGCCCGGCA	472998	92	2-10-2	2320	2333	74
15807	15820	GGTGCCAGGACGG	492440	89	2-10-2	2864	2877	178

Example 6: Modified antisense oligonucleotides comprising cEt modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. Also tested was ISIS 407939, a 5-10-5 MOE gapmer targeting human Factor VII, which was described in an earlier publication (WO 2009/061851). ISIS 472998 and ISIS 473046, described in the Examples above were also included in the screen.

The newly designed modified antisense oligonucleotides in Table 7 were designed as 2-10-2 cEt gapmers. The 2-10-2 cEt gapmers are 14 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising two nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosine residues throughout each oligonucleotide are 5-methylcytosines.

Each oligonucleotide listed in Table 7 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Activity of the newly designed gapmers was compared to ISIS 407939. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 757 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Table 7. Each of the

newly designed antisense oligonucleotides provided in Table 7 achieved greater than 67% inhibition and, therefore, are more active than 407939.

Table 7

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

5

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	ISIS No	% inhibition	Motif	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	SEQ ID NO
15255	15274	TGCAGCCCGGCACCCAGCGA	407939	67	5-10-5	2312	2331	72
15263	15276	TGTGCAGCCCGGCA	472998	85	2-10-2	2320	2333	74
11840	11853	ATGGTCAGGGCTGG	473046	79	2-10-2	n/a	n/a	35
5513	5526	CGAGGCGCGGCCCC	492651	77	2-10-2	n/a	n/a	179
5514	5527	CCGAGGCGCGGCCCC	492652	84	2-10-2	n/a	n/a	180
5558	5571	GTCTCCGCGCGCCA	492658	87	2-10-2	n/a	n/a	181
8608	8621	GCTGTGAGAATACA	492725	74	2-10-2	n/a	n/a	182
8644	8657	GAAACTGTTGGCCA	492730	78	2-10-2	n/a	n/a	183
8645	8658	AGAAACTGTTGGCC	492731	72	2-10-2	n/a	n/a	184
8862	8875	TGGGTGACCACACA	492784	72	2-10-2	n/a	n/a	185
9358	9371	GGTTGTGCACCCTG	492816	70	2-10-2	n/a	n/a	186
9360	9373	CAGGTTGTGCACCC	492818	73	2-10-2	n/a	n/a	187
9599	9612	AGTTTACCAAGCGG	492877	83	2-10-2	n/a	n/a	188
9600	9613	AAGTTTACCAAGCG	492878	79	2-10-2	n/a	n/a	189
9940	9953	CCTCTGGACACCGG	492913	73	2-10-2	n/a	n/a	190
9941	9954	ACCTCTGGACACCG	492914	82	2-10-2	n/a	n/a	191
9960	9973	GTGATTGAGCCCTG	492928	76	5-10-5	n/a	n/a	192
10069	10082	GGTCTAGCTGACAA	492938	80	2-10-2	n/a	n/a	193
10385	10398	GGATGCACACCAGG	492991	91	2-10-2	n/a	n/a	194
10386	10399	AGGATGCACACCAG	492992	73	2-10-2	n/a	n/a	195
11144	11157	GGTGTCTATCTGGGA	493087	81	2-10-2	n/a	n/a	196
11283	11296	CTGTGCTCTGGCC	493114	80	2-10-2	n/a	n/a	197
11545	11558	GGAAGTGCAGCCCA	493178	86	2-10-2	n/a	n/a	198
11546	11559	TGGAAGTGCAGCCC	493179	69	2-10-2	n/a	n/a	199
11703	11716	GTTGTTTTGATCCC	493182	79	2-10-2	n/a	n/a	200
11838	11851	GGTCAGGGCTGGTT	493195	71	2-10-2	n/a	n/a	201
11847	11860	GGAGACAATGGTCA	493201	86	2-10-2	n/a	n/a	202
11848	11861	AGGAGACAATGGTC	493202	76	2-10-2	n/a	n/a	203
12406	12419	TCTCTGCACAGGGT	493255	80	2-10-2	n/a	n/a	204
12506	12519	GATCCAATGCTCCT	493291	84	2-10-2	n/a	n/a	205
12507	12520	TGATCCAATGCTCC	493292	90	2-10-2	n/a	n/a	206
12511	12524	GCTTTGATCCAATG	493296	82	2-10-2	n/a	n/a	207
12513	12526	TAGCTTTGATCCAA	493298	77	2-10-2	n/a	n/a	208
12514	12527	ATAGCTTTGATCCA	493299	76	2-10-2	n/a	n/a	209
12521	12534	TCTTCACATAGCTT	493304	77	2-10-2	n/a	n/a	210

12554	12567	TCGCTGTGAGATTT	493312	75	2-10-2	n/a	n/a	211
12692	12705	GGCATTGCACAATT	493333	76	2-10-2	n/a	n/a	212

Example 7: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Antisense oligonucleotides from the studies above, exhibiting *in vitro* inhibition of Factor VII mRNA were selected and tested at various doses in Hep3B cells. Also tested was ISIS 407939, a 5-10-5 MOE gapmer, which was described in an earlier publication (WO 2009/061851).

5 Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.67 μ M, 2.00 μ M, 1.11 μ M, and 6.00 μ M concentrations of antisense oligonucleotide, as specified in Table 8. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human Factor VII primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were
10 adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 8. As illustrated in Table 8, Factor VII mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. The data also confirms that many of the newly designed oligonucleotides
15 achieved an IC_{50} of less than 0.7 μ M and, therefore, are more potent than ISIS 407939.

Table 8

Dose-dependent antisense inhibition (%) of human Factor VII in Hep3B cells using electroporation

ISIS No	666.6667 nM	2000.0 nM	6000.0 nM	IC_{50} (μ M)
407939	47	68	85	0.7
457851	60	80	93	<0.6
472925	62	86	95	<0.6
472926	66	77	85	<0.6
472935	54	84	94	<0.6
472958	66	82	88	<0.6
472959	64	81	93	<0.6
472970	72	87	86	<0.6
472976	78	92	97	<0.6
472994	79	92	96	<0.6
472995	61	82	93	<0.6
472996	73	91	95	<0.6
472998	63	90	95	<0.6
473019	55	80	86	<0.6
473020	61	76	85	<0.6
473046	61	80	94	<0.6
473055	55	84	94	<0.6
492104	53	76	88	<0.6
492105	62	80	90	<0.6

492189	57	80	92	<0.6
492194	57	83	91	<0.6
492195	58	81	95	<0.6
492196	62	86	95	<0.6
492205	62	87	95	<0.6
492215	60	78	89	<0.6
492221	63	76	92	<0.6
492234	51	74	91	0.5
492276	50	56	95	0.8
492277	58	73	81	<0.6
492306	61	75	84	<0.6
492317	59	80	93	<0.6
492329	59	70	89	<0.6
492331	69	87	95	<0.6
492333	47	70	85	0.7
492334	57	77	90	<0.6
492390	72	88	95	<0.6
492399	68	91	96	<0.6
492401	68	89	95	<0.6
492404	65	87	94	<0.6
492405	44	81	90	0.7
492406	65	82	92	<0.6
492440	50	70	89	0.6
492465	16	80	79	1.4
492467	58	77	92	<0.6
492492	45	80	94	0.7
492494	63	82	93	<0.6
492503	55	81	93	<0.6
492530	70	86	90	<0.6
492534	67	85	91	<0.6
492536	54	81	89	<0.6
492541	54	71	85	<0.6
492545	59	78	89	<0.6
492566	59	84	85	<0.6
492571	52	81	89	<0.6
492572	67	83	90	<0.6
492573	69	83	92	<0.6
492574	65	82	91	<0.6
492575	72	83	91	<0.6
492593	61	78	90	<0.6
492617	62	80	93	<0.6
492618	47	79	94	0.6
492619	54	82	95	<0.6
492621	44	85	92	0.6
492651	53	66	91	0.6
492652	61	78	88	<0.6

492658	59	79	88	<0.6
492725	43	84	89	0.6
492730	51	87	93	0.4
492731	46	82	90	0.6
492784	56	88	96	<0.6
492816	68	89	97	<0.6
492818	64	84	96	<0.6
492877	67	91	93	<0.6
492878	80	89	93	<0.6
492913	53	87	92	<0.6
492914	75	89	96	<0.6
492928	60	83	94	<0.6
492938	70	90	92	<0.6
492991	67	93	99	<0.6
492992	0	82	95	2.1
493087	54	81	90	<0.6
493114	50	73	90	0.6
493178	71	88	96	<0.6
493179	47	82	95	0.6
493182	79	87	91	<0.6
493195	55	78	90	<0.6
493201	87	93	96	<0.6
493202	68	89	94	<0.6
493255	57	79	93	<0.6
493291	57	87	93	<0.6
493292	70	89	93	<0.6
493296	35	84	91	0.9
493298	57	84	92	<0.6
493299	65	84	93	<0.6
493304	68	86	94	<0.6
493312	53	82	91	<0.6
493333	66	84	87	<0.6

Example 8: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Additional antisense oligonucleotides from the studies above, exhibiting *in vitro* inhibition of Factor VII mRNA were selected and tested at various doses in Hep3B cells. Also tested was ISIS 407939, a 5-10-5 MOE gapmer, which was described in an earlier publication (WO 2009/061851).

Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.67 μ M, 2.00 μ M, 1.11 μ M, and 6.00 μ M 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 VII mRNA levels were measured by quantitative real-time PCR. Human Factor VII primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were

adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 9. As illustrated in Table 9, Factor VII mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. The data also confirms that each of the newly designed oligonucleotides achieved an IC₅₀ of less than 0.6 μ M and, therefore, are more potent than ISIS 407939.

Table 9

Dose-dependent antisense inhibition (%) of human Factor VII in Hep3B cells using electroporation

ISIS No	0.67 μ M	2.00 μ M	6.00 μ M	IC ₅₀ (μ M)
407939	52	71	86	0.6
472983	49	83	97	0.5
472984	51	82	95	0.5
472991	49	82	95	0.5
472998	59	88	96	<0.6
492365	74	91	96	<0.6
492377	56	76	91	<0.6
492380	63	79	95	<0.6
492384	67	84	94	<0.6
492388	69	87	97	<0.6
492389	62	90	96	<0.6
492391	56	84	94	<0.6
492398	63	80	95	<0.6
492403	58	81	91	<0.6

10 **Example 9: Modified antisense oligonucleotides comprising MOE modifications targeting human coagulation Factor VII**

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. Also tested were ISIS 403052, ISIS 407939, ISIS 416446, ISIS 416472, ISIS 416507, ISIS 416508, ISIS 422087, ISIS 422096, ISIS 422130, and ISIS 422142 which were described in an earlier publication (WO 2009/061851), incorporated herein by reference. ISIS 490149, ISIS 490197, ISIS 490209, ISIS 490275, ISIS 490277, and ISIS 490424, described in the Examples above, were also included in the screen.

The newly designed modified antisense oligonucleotides in Table 10 were designed as 3-10-4 MOE gapmers. These gapmers are 17 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) with wing segments. The 5' wing segment comprises three MOE nucleosides and the 3' wing comprises four MOE nucleosides. The

internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

Each gapmer listed in Table 10 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Activity of the newly designed oligonucleotides was compared to ISIS 403052, ISIS 407939, ISIS 416446, ISIS 416472, ISIS 416507, ISIS 416508, ISIS 422087, ISIS 422096, ISIS 422130, and ISIS 422142. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 272 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Table 10. Several of the newly designed antisense oligonucleotides provided in Table 10 are more active than antisense oligonucleotides from the previous publication.

Table 10

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	ISIS No	% inhibition	Motif	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	SEQ ID NO
n/a	n/a	GGCACACTGGTCCCCATCAC	403052	51	5-10-5	299	318	82
15255	15274	TGCAGCCCGGCACCCAGCGA	407939	78	5-10-5	2312	2331	72
11024	11043	GGTCACTGCAGTACTGCTCA	416446	70	5-10-5	459	478	103
14348	14367	CAGCCTTGGCTTTCTCTCCA	416472	79	5-10-5	1405	1424	111
4847	4866	GGTTACTGAGCGCGGAAGAA	416507	84	5-10-5	n/a	n/a	97
4873	4892	CGAGTTCTGCAGGAGCGGCC	416508	80	5-10-5	n/a	n/a	100
15192	15211	CATGCATGGTGATGCTTCTG	422087	89	5-10-5	2249	2268	121
11839	11858	AGACAATGGTCAGGGCTGGT	422096	78	5-10-5	n/a	n/a	219
14708	14727	GCCTGAGGCCAGCAGATCAC	422130	81	5-10-5	1765	1784	225
4872	4891	GAGTTCTGCAGGAGCGGCCT	422142	84	5-10-5	n/a	n/a	99
1383	1402	CCTGTGCCTGGATGCTGGTT	490275	77	5-10-5	n/a	n/a	90
1383	1399	GTGCCTGGATGCTGGTT	513462	79	3-10-4	n/a	n/a	213
1384	1400	TGTGCCTGGATGCTGGT	513463	81	3-10-4	n/a	n/a	214

1385	1404	CTCCTGTGCCTGGATGCTGG	490277	74	5-10-5	n/a	n/a	91
2490	2506	GCATCAGAAAAGCTCTC	513487	83	3-10-4	n/a	n/a	215
4850	4866	GGTTACTGAGCGCGGAA	513504	81	3-10-4	n/a	n/a	216
4873	4889	GTTCTGCAGGAGCGGCC	513507	86	3-10-4	n/a	n/a	217
4874	4890	AGTTCTGCAGGAGCGGC	513508	85	3-10-4	n/a	n/a	218
4939	4958	GGAGGGACGACCTTTGCTGG	490424	69	5-10-5	n/a	n/a	101
12505	12524	GCTTTGATCCAATGCTCCTG	491122	87	5-10-5	n/a	n/a	220
12631	12647	GCTGCTCAGACCTGGCT	513642	79	3-10-4	n/a	n/a	221
14232	14251	GGAGACCCTGGTGTACACCC	490149	71	5-10-5	1289	1308	109
14612	14628	GCCCCCTCTGTCCAGCGC	513419	90	3-10-4	1669	1685	222
14613	14629	TGCCCCCTCTGTCCAGCG	513420	89	3-10-4	1670	1686	223
14614	14630	CTGCCCCCTCTGTCCAGC	513421	88	3-10-4	1671	1687	224
15130	15149	GTGAGAGCTAAACAACCGCC	490197	77	5-10-5	2187	2206	116
15182	15198	GCTTCTGAATTGTCTGA	513446	89	3-10-4	2239	2255	226
15183	15199	TGCTTCTGAATTGTCTG	513447	83	3-10-4	2240	2256	227
15184	15203	GTGATGCTTCTGAATTGTCT	490209	79	5-10-5	2241	2260	118
15191	15207	CATGGTGATGCTTCTGA	513454	84	3-10-4	2248	2264	228
15192	15208	GCATGGTGATGCTTCTG	513455	92	3-10-4	2249	2265	229
15193	15209	TGCATGGTGATGCTTCT	513456	89	3-10-4	2250	2266	230
15194	15210	ATGCATGGTGATGCTTC	513457	83	3-10-4	2251	2267	231

Example 10: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Antisense oligonucleotides from the studies above, exhibiting *in vitro* inhibition of Factor VII mRNA, were selected and tested at various doses in Hep3B cells. Also tested were ISIS 403052, ISIS 407643, ISIS 407935, ISIS 407936, ISIS 407939, ISIS 416446, ISIS 416459, ISIS 416472, ISIS 416507, ISIS 416508, ISIS 416549, ISIS 422086, ISIS 422087, ISIS 422130, ISIS and 422142, 5-10-5 MOE gapmers targeting human Factor VII, which were described in an earlier publication (WO 2009/061851).

Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.625 μ M, 1.25 μ M, 2.50 μ M, 5.00 μ M and 10.00 μ M concentrations of antisense oligonucleotide, as specified in Table 11. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human Factor VII primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 11. As illustrated in Table 11, Factor VII mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. The data also confirms that several of the newly designed oligonucleotides are more potent than oligonucleotides from the previous publication.

Table 11

Dose-dependent antisense inhibition (%) of human Factor VII in Hep3B cells using electroporation

ISIS No	0.625 μM	1.25 μM	2.50 μM	5.00 μM	10.00 μM	IC ₅₀ (μM)
403052	21	35	63	82	89	1.9
407643	29	46	67	83	90	1.4
407935	52	68	80	89	91	<0.6
407936	31	51	62	78	84	1.4
407939	30	61	74	83	88	1.0
416446	37	53	64	76	83	1.2
416459	51	76	83	90	92	<0.6
416472	37	52	66	78	85	1.2
416507	45	68	82	87	90	0.7
416508	33	56	74	84	89	1.1
416549	57	71	78	82	85	<0.6
422086	46	67	77	89	92	0.7
422087	50	69	74	86	91	0.6
422130	32	65	78	92	93	0.9
422142	59	73	84	86	88	<0.6
490103	52	57	66	83	88	0.9
490149	34	58	71	85	91	1.0
490196	26	59	66	79	84	1.3
490197	39	63	74	81	90	0.8
490208	44	70	76	83	88	0.6
490275	36	58	76	85	89	1.0
490277	37	63	73	87	87	0.8
490279	40	54	72	83	89	1.0
490323	49	68	79	86	90	<0.6
490368	39	62	76	86	91	0.8
490396	36	53	69	80	87	1.1
490424	45	65	69	76	82	0.6
490803	57	74	85	89	92	<0.6
513419	60	71	85	95	96	<0.6
513420	37	69	79	94	96	0.7
513421	46	64	84	95	97	0.6
513446	47	81	88	95	96	<0.6
513447	56	74	81	92	96	<0.6
513454	50	77	82	93	95	<0.6
513455	74	82	91	96	96	<0.6
513456	66	80	88	94	95	<0.6
513457	54	67	80	87	89	<0.6
513462	49	72	84	87	89	<0.6
513463	36	62	76	85	89	0.9
513487	42	56	73	87	93	0.9

513504	47	65	81	90	91	0.6
513505	39	50	78	85	92	1.0
513507	52	73	83	89	93	<0.6
513508	56	78	85	91	94	<0.6

Example 11: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Additional antisense oligonucleotides from the studies above, exhibiting *in vitro* inhibition of Factor VII mRNA, were tested at various doses in Hep3B cells. Also tested were ISIS 407935, ISIS 407939, ISIS 416446, ISIS 416472, ISIS 416507, ISIS 416549, ISIS 422086, ISIS 422087, ISIS 422096, and ISIS 422142, 5-10-5 MOE gapmers targeting human Factor VII, which were described in an earlier publication (WO 2009/061851).

Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.3125 μ M, 0.625 μ M, 1.25 μ M, 2.50 μ M, 5.00 μ M and 10.00 μ M concentrations of antisense oligonucleotide, as specified in Table 12. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human Factor VII primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 12. As illustrated in Table 12, Factor VII mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. The data also confirms that several of the newly designed oligonucleotides are more potent than oligonucleotides from the previous publication.

Table 12

Dose-dependent antisense inhibition (%) of human Factor VII in Hep3B cells using electroporation

ISIS No	0.3125 μ M	0.625 μ M	1.250 μ M	2.500 μ M	5.000 μ M	10.000 μ M	IC ₅₀ (μ M)
407935	30	49	75	86	91	94	0.6
407939	30	48	61	78	85	90	0.8
416446	27	52	63	75	85	90	0.7
416472	38	51	72	83	88	94	0.5
416507	58	81	76	84	89	92	<0.3
416549	52	67	75	81	88	89	0.3
422086	48	49	68	78	86	91	0.5
422087	30	56	66	83	72	92	0.6
422096	47	63	70	77	83	85	<0.3
422142	69	85	87	85	89	91	<0.3
490103	52	57	68	78	87	93	0.4
490149	33	64	62	77	86	93	0.5

490197	38	46	60	75	87	93	0.7
490208	46	62	73	83	88	91	0.4
490209	40	54	72	79	85	94	0.5
490275	52	61	67	78	85	91	0.3
490277	33	59	77	79	91	94	0.5
490323	43	61	72	69	84	87	0.4
490368	50	64	78	83	90	92	<0.3
490396	46	64	68	84	84	90	0.3
490424	24	47	58	72	76	82	1.0
490803	45	60	70	84	88	89	0.3
513419	32	53	76	88	93	95	0.5
513420	35	59	72	82	94	97	0.5
513421	46	67	78	86	94	96	<0.3
513446	26	61	77	89	91	97	0.5
513447	22	48	60	82	91	95	0.8
513454	25	59	76	86	94	96	0.5
513455	60	73	85	89	95	96	<0.3
513456	49	60	81	88	94	95	<0.3
513457	43	50	72	77	87	92	0.5
513462	25	48	58	76	83	88	0.8
513463	22	45	66	73	85	88	0.9
513487	41	56	65	79	86	90	0.4
513504	19	48	63	76	87	92	0.9
513505	11	21	54	73	85	90	1.4
513507	47	55	72	82	90	91	0.3
513508	31	59	74	85	92	93	0.5
513642	43	55	67	80	88	92	0.4

Example 12: Tolerability of MOE gapmers targeting human Factor VII in BALB/c mice

BALB/c mice are a multipurpose mice model, frequently utilized for safety and efficacy testing. The mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Groups of male BALB/c mice were injected subcutaneously twice a week for 3 weeks with 50 mg/kg of ISIS 407935, ISIS 416472, ISIS 416549, ISIS 422086, ISIS 422087, ISIS 422096, ISIS 422142, ISIS 490103, ISIS 490149, ISIS 490196, ISIS 490208, ISIS 490209, ISIS 513419, ISIS 513420, ISIS 513421, ISIS 513454, ISIS 513455, ISIS 513456, ISIS 513457, ISIS 513462, ISIS 513463, ISIS 513487, ISIS 513504, ISIS 513508, and ISIS 513642. One group of male BALB/c mice was injected subcutaneously twice a week for 3 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, bilirubin, albumin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS oligonucleotides that caused an increase in the levels of transaminases between three times and seven times the ULN were deemed tolerable. Based on these criteria, ISIS 407935, ISIS 416472, ISIS 416549, ISIS 422087, ISIS 422096, ISIS 490103, ISIS 490196, ISIS 490208, ISIS 513454, ISIS 513455, ISIS 513456, ISIS 513457, ISIS 513487, ISIS 513504, and ISIS 513508 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 422086, ISIS 490209, ISIS 513419, ISIS 513420, and ISIS 513463 were considered tolerable in terms of liver function.

Example 13: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Additional antisense oligonucleotides from the studies above, exhibiting *in vitro* inhibition of Factor VII mRNA were selected and tested at various doses in Hep3B cells. Also tested was ISIS 407939, a 5-10-5 MOE gapmer, which was described in an earlier publication (WO 2009/061851).

Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.074 μ M, 0.222 μ M, 0.667 μ M, 2.000 μ M, and 6.000 μ M concentrations of antisense oligonucleotide, as specified in Table 13. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human Factor VII primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 13. As illustrated in Table 13, Factor VII mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. Many of the newly designed antisense oligonucleotides provided in Table 13 achieved an IC₅₀ of less than 0.9 μ M and, therefore, are more potent than ISIS 407939.

Table 13

Dose-dependent antisense inhibition (%) of human Factor VII in Hep3B cells using electroporation

ISIS No	0.074 μ M	0.222 μ M	0.667 μ M	2.000 μ M	6.000 μ M	IC ₅₀ (μ M)
407939	2	17	53	70	87	0.9
472970	17	47	75	92	95	0.3
472988	0	8	21	54	92	1.4
472996	18	59	74	93	95	0.2
473244	91	95	97	99	99	<0.07

473286	6	53	85	92	98	0.3
473359	2	3	20	47	67	2.6
473392	71	85	88	92	96	<0.07
473393	91	96	97	98	99	<0.07
473547	85	88	93	97	98	<0.07
473567	0	25	66	88	95	0.7
473589	8	47	79	94	99	0.3
482814	23	68	86	93	96	0.1
482815	6	48	65	90	96	0.4
482963	3	68	85	94	96	0.2
483241	14	33	44	76	93	0.6
483261	14	21	41	72	88	0.7
483290	0	1	41	69	92	1.0
483414	8	1	36	76	91	0.9
483415	0	40	52	84	94	0.6
484559	26	51	78	87	97	0.2
484713	6	5	53	64	88	0.9

Example 14: Modified antisense oligonucleotides comprising cEt and MOE modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. Also tested was ISIS 407939, a 5-10-5 MOE gapmer targeting human Factor VII, which was described in an earlier publication (WO 2009/061851). ISIS 472998, ISIS 492878, ISIS 493201, and 493182, which are 2-10-2 cEt gapmers described in the Examples above, were also included in the screen.

The newly designed modified antisense oligonucleotides and their motifs are described in Table 14. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The ‘Sugar Chemistry’ column provides the sugar modifications throughout each oligonucleotide: ‘d’ indicates a 2’-deoxynucleoside, ‘k’ indicates a constrained ethyl (cEt) nucleoside, and ‘e’ indicates a 2’-O-methoxyethyl nucleoside. The ‘Sequence’ column provides the nucleobase sequence for each SEQ ID NO..

Each oligonucleotide listed in Table 14 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. “Start site” indicates the 5’-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. “Stop site” indicates the 3’-most nucleoside to which the oligonucleotide is targeted human gene sequence. ‘n/a’ indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Activity of newly designed oligonucleotides was compared to ISIS 407939. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. A total of 685 oligonucleotides were tested. Only those oligonucleotides which were selected for further studies are shown in Table 14. Many of the newly designed antisense oligonucleotides provided in Table 14 achieved greater than 68% inhibition and, therefore, are more active than ISIS 407939.

Table 14

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	% inhibition	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Sugar Chemistry
15255	15274	TGCAGCCCGGCACCCAGCGA	72	407939	68	2312	2331	eeeeeddddddddeeeee
9600	9613	AAGTTTACCAAGCG	189	492878	73	n/a	n/a	kkdddddddddkk
11703	11716	GTTGTTTTGATCCC	200	493182	80	n/a	n/a	kkdddddddddkk
11847	11860	GGAGACAATGGTCA	202	493201	84	n/a	n/a	kkdddddddddkk
15263	15276	TGTGCAGCCCGGCA	74	472998	91	2320	2333	kkdddddddddkk
1382	1397	GCCTGGATGCTGGTTT	23	515640	75	n/a	n/a	eeddddddddddkkk
1383	1398	TGCCTGGATGCTGGTT	232	515637	77	n/a	n/a	eeddddddddddkkk
2499	2514	GCAGATTGTCATCAGA	233	515554	72	n/a	n/a	eeddddddddddkkk
4851	4866	GGTTACTGAGCGCGGA	234	515406	80	n/a	n/a	kkkddddddddddeee
4851	4866	GGTTACTGAGCGCGGA	234	515558	81	n/a	n/a	eeddddddddddkkk
4872	4887	TCTGCAGGAGCGGCCT	235	515407	88	n/a	n/a	kkkddddddddddeee
4873	4888	TTCTGCAGGAGCGGCC	236	515408	85	n/a	n/a	kkkddddddddddeee
5374	5389	GACCTCGCGCGGATCC	237	515422	86	n/a	n/a	kkkddddddddddeee
5512	5527	CCGAGGCGCGGCCCT	238	515423	90	n/a	n/a	kkkddddddddddeee
5512	5527	CCGAGGCGCGGCCCT	238	515575	84	n/a	n/a	eeddddddddddkkk
5513	5528	TCCGAGGCGCGGCCCC	239	515424	87	n/a	n/a	kkkddddddddddeee
8643	8658	AGAAACTGTTGGCCAC	240	515432	78	n/a	n/a	kkkddddddddddeee
8644	8659	AAGAAACTGTTGGCCA	241	515433	71	n/a	n/a	kkkddddddddddeee
8655	8670	AGTGATTGCTGAAGAA	242	515434	76	n/a	n/a	kkkddddddddddeee
9169	9184	GGCACACTGGTCCCCA	243	515334	85	303	318	kkkddddddddddeee
9170	9185	AGGCACACTGGTCCCC	88	515649	61	304	319	eeddddddddddkkk
9225	9240	CAGATATAGGACTGGA	244	515338	86	359	374	kkkddddddddddeee
9359	9374	CCAGGTTGTGCACCCT	245	515438	76	n/a	n/a	kkkddddddddddeee
9453	9468	CCTGTCAAAGACCTCA	246	515439	75	n/a	n/a	kkkddddddddddeee
10383	10398	GGATGCACACCAGGGC	247	516003	87	n/a	n/a	kkddddddddddeeee
11016	11031	ACTGCTCACAGCCGCC	77	515647	60	451	466	eeddddddddddkkk

11839	11854	AATGGTCAGGGCTGGT	34	515639	78	n/a	n/a	eeedddddddddkkk
12127	12142	GGGTTTGCTGGCATT	248	515648	36	597	612	eeedddddddddkkk
12633	12648	AGCTGCTCAGACCTGG	39	515641	69	n/a	n/a	eeedddddddddkkk
13741	13756	GTGCTCGCTGAGGTCG	44	515650	76	798	813	eeedddddddddkkk
13742	13757	CGTGCTCGCTGAGGTC	249	515354	87	799	814	kkkdddddddddeee
14077	14092	GAACATGTACTCCGTG	250	515926	87	1134	1149	kkkdddddddddeeee
14094	14109	TCCGAGTAGCCGGCAC	251	515366	87	1151	1166	kkkdddddddddeeee
14243	14258	TGTACTGGGAGACCCT	252	515642	58	1300	1315	eeedddddddddkkk
14612	14627	CCCCTCTGTCCAGCGC	53	515643	81	1669	1684	eeedddddddddkkk
15130	15145	GAGCTAAACAACCGCC	253	515944	84	2187	2202	kkkdddddddddeeee
15131	15146	AGAGCTAAACAACCGC	254	515380	90	2188	2203	kkkdddddddddeeee
15131	15146	AGAGCTAAACAACCGC	254	515532	83	2188	2203	eeedddddddddkkk
15131	15146	AGAGCTAAACAACCGC	254	515945	85	2188	2203	kkkdddddddddeeee
15132	15147	GAGAGCTAAACAACCG	255	515381	82	2189	2204	kkkdddddddddeeee
15183	15198	GCTTCTGAATTGTCTG	256	515382	95	2240	2255	kkkdddddddddeeee
15183	15198	GCTTCTGAATTGTCTG	256	515948	94	2240	2255	kkkdddddddddeeee
15185	15200	ATGCTTCTGAATTGTC	257	515949	87	2242	2257	kkkdddddddddeeee
15186	15201	GATGCTTCTGAATTGT	258	515384	89	2243	2258	kkkdddddddddeeee
15190	15205	TGGTGATGCTTCTGAA	65	515635	82	2247	2262	eeedddddddddkkk
15191	15206	ATGGTGATGCTTCTGA	67	515638	90	2248	2263	eeedddddddddkkk
15192	15207	CATGGTGATGCTTCTG	259	515386	92	2249	2264	kkkdddddddddeeee
15192	15207	CATGGTGATGCTTCTG	259	515951	84	2249	2264	kkkdddddddddeeee
15193	15208	GCATGGTGATGCTTCT	260	515387	78	2250	2265	kkkdddddddddeeee
15193	15208	GCATGGTGATGCTTCT	260	515952	89	2250	2265	kkkdddddddddeeee
15194	15209	TGCATGGTGATGCTTC	69	515636	90	2251	2266	eeedddddddddkkk
15196	15211	CATGCATGGTGATGCT	261	515388	84	2253	2268	kkkdddddddddeeee

Example 15: Tolerability of modified oligonucleotides having deoxy, MOE, and cEt modifications targeting human Factor VII in BALB/c mice

BALB/c mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Additionally, newly designed antisense oligonucleotides were also added to this screen. The newly designed modified antisense oligonucleotides and their motifs are described in Table 15. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The ‘Sugar Chemistry’ column provides the sugar modifications throughout each oligonucleotide: ‘d’ indicates a 2’-deoxynucleoside, ‘k’ indicates a constrained ethyl (cEt) nucleoside, and ‘e’ indicates a 2’-O-methoxyethyl nucleoside. The ‘Sequence’ column provides the nucleobase sequence for each SEQ ID NO.

Each oligonucleotide listed in Table 15 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Table 15
Modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Sugar Chemistry
1147	1162	GATGAAATCTCTGCAG	21	516044	36	51	eeddddddskkk
1154	1169	AGACCATGATGAAATC	22	516045	43	58	eeddddddskkk
2369	2384	TGGAGCGGTCACCTCC	26	516058	n/a	n/a	eeddddddskkk
4717	4732	AGGAGGCTGAGGATGC	27	516059	n/a	n/a	eeddddddskkk
4871	4886	CTGCAGGAGCGGCCTA	28	516060	n/a	n/a	eeddddddskkk
6411	6426	CGTATTTTCTGATGTG	29	516061	n/a	n/a	eeddddddskkk
6642	6657	GAGGTGACCCGTGAGC	30	516062	n/a	n/a	eeddddddskkk
12141	12156	ACAATTCGGCCTTGGG	37	516046	611	626	eeddddddskkk
12629	12644	GCTCAGACCTGGCTCT	38	516063	n/a	n/a	eeddddddskkk
12631	12646	CTGCTCAGACCTGGCT	89	516064	n/a	n/a	eeddddddskkk
12634	12649	AAGCTGCTCAGACCTG	262	516065	n/a	n/a	eeddddddskkk
12635	12650	AAAGCTGCTCAGACCT	263	516066	n/a	n/a	eeddddddskkk
12842	12857	CCACCCAGATGGTGTT	41	516047	715	730	eeddddddskkk
12863	12878	CGAAACAGTGGGCCGC	42	516048	736	751	eeddddddskkk
13760	13775	GGCTCTGCTCATCCCC	81	516049	817	832	eeddddddskkk
13988	14003	CCATGAGCTCCAGGGC	45	516050	1045	1060	eeddddddskkk
14079	14094	CAGAACATGTACTCCG	48	516051	1136	1151	eeddddddskkk
14092	14107	CGAGTAGCCGGCACAG	49	516052	1149	1164	eeddddddskkk
14128	14143	TCCACTGTCCCCCTTG	50	515652	1185	1200	eeddddddskkk
14231	14246	CCCTGGTGTACACCCC	264	508039	1288	1303	eeddddddskkk
14232	14247	ACCCTGGTGTACACCC	265	516053	1289	1304	eeddddddskkk
14708	14723	GAGGCCAGCAGATCAC	76	515654	1765	1780	eeddddddskkk
14713	14728	AGCCTGAGGCCAGCAG	77	515656	1770	1785	eeddddddskkk
15097	15112	ATGGAGTCAGCATCGG	57	516054	2154	2169	eeddddddskkk
15128	15143	GCTAAACAACCGCCTT	59	516055	2185	2200	eeddddddskkk
15164	15179	TGAAGATGATAATGGA	61	515655	2221	2236	eeddddddskkk
15181	15196	TTCTGAATTGTCTGAA	63	516056	2238	2253	eeddddddskkk
15188	15203	GTGATGCTTCTGAATT	64	516057	2245	2260	eeddddddskkk
15195	15210	ATGCATGGTGATGCTT	71	515653	2252	2267	eeddddddskkk
15262	15277	CTGTGCAGCCCGGCAC	73	515657	2319	2334	eeddddddskkk

Treatment

Groups of 4-6-week old male BALB/c mice were injected subcutaneously twice a week for 3 weeks with 25 mg/kg of ISIS 457851, ISIS 515635, ISIS 515636, ISIS 515637, ISIS 515638, ISIS 515639, ISIS 515640, ISIS 515641, ISIS 515642, ISIS 515643, ISIS 515647, ISIS 515648, ISIS 515649, ISIS 515650, ISIS 515652, ISIS 515653, ISIS 515654, ISIS 515655, ISIS 515656, ISIS 515657, ISIS 516044, ISIS 516045, ISIS 516046, ISIS 516047, ISIS 516048, ISIS 516049, ISIS 516050, ISIS 516051, ISIS 516052, ISIS 516053, ISIS 516054, ISIS 516055, ISIS 516056, ISIS 516057, ISIS 516058, ISIS 516059, ISIS 516060, ISIS 516061, ISIS 516062, ISIS 516063, ISIS 516064, ISIS 516065, or ISIS 516066. One group of 4-6-week old male BALB/c mice was injected subcutaneously twice a week for 3 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, bilirubin, albumin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS oligonucleotides that caused an increase in the levels of transaminases between three times and seven times the ULN were deemed tolerable. Based on these criteria, ISIS 515636, ISIS 515639, ISIS 515641, ISIS 515642, ISIS 515648, ISIS 515650, ISIS 515652, ISIS 515653, ISIS 515655, ISIS 515657, ISIS 516044, ISIS 516045, ISIS 516047, ISIS 516048, ISIS 516051, ISIS 516052, ISIS 516053, ISIS 516055, ISIS 516056, ISIS 516058, ISIS 516059, ISIS 516060, ISIS 516061, ISIS 516062, ISIS 516063, ISIS 516064, ISIS 516065, and ISIS 516066 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 457851, ISIS 515635, ISIS 515637, ISIS 515638, ISIS 515643, ISIS 515647, ISIS 515649, ISIS 515650, ISIS 515652, ISIS 515654, ISIS 515656, ISIS 516056, and ISIS 516057 were considered tolerable in terms of liver function.

Example 16: Efficacy of modified antisense oligonucleotides comprising MOE and cEt modifications targeting human Factor VII in transgenic mice

Transgenic mice were developed at Taconic Farms Inc. harboring a Factor VII genomic DNA fragment. The mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for efficacy.

Treatment

Groups of 3-4 male and female transgenic mice were injected subcutaneously twice a week for 3 weeks with 10 mg/kg of ISIS 457851, ISIS 515636, ISIS 515639, ISIS 515653, ISIS 516053, ISIS 516065, or ISIS 516066. One group of mice was injected subcutaneously twice a week for 3 weeks with control

oligonucleotide, ISIS 141923 (CCTTCCCTGAAGGTTCTCTCC, 5'-10-5 MOE gapmer with no known murine target, SEQ ID NO: 266). One group of mice was injected subcutaneously twice a week for 3 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

5 *RNA Analysis*

RNA was extracted from plasma for real-time PCR analysis of Factor VII, using primer probe set RTS2927 (described hereinabove in Example 1). The mRNA levels were normalized using RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to control. As shown in Table 16, each of the antisense oligonucleotides achieved reduction of human Factor VII mRNA expression over the PBS control. Treatment with the control oligonucleotide did not achieve reduction in Factor VII levels, as expected.

Table 16
Percent inhibition of Factor VII mRNA in transgenic mice

ISIS No	% inhibition
141923	0
457851	76
515636	66
515639	49
515653	78
516053	72
516065	59
516066	39

Protein Analysis

15 Plasma protein levels of Factor VII were estimated using a Zymutest FVII ELISA kit (Hyphen Bio-Med cat# ARK036A). Results are presented as percent inhibition of Factor VII, relative to control. As shown in Table 17, several antisense oligonucleotides achieved reduction of human Factor VII protein expression over the PBS control.

Table 17
Percent inhibition of Factor VII protein levels in transgenic mice

ISIS No	% inhibition
141923	0
457851	64
515636	68
515639	46
515653	0
516053	19

516065	0
516066	7

Example 17: Efficacy of modified antisense oligonucleotides comprising MOE and cEt modifications targeting human Factor VII in transgenic mice

Transgenic mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for efficacy.

5 *Treatment*

Groups of 2-4 male and female transgenic mice were injected subcutaneously twice a week for 3 weeks with 5 mg/kg of ISIS 407935, ISIS 416472, ISIS 416549, ISIS 422087, ISIS 422096, ISIS 473137, ISIS 473244, ISIS 473326, ISIS 473327, ISIS 473359, ISIS 473392, ISIS 473393, ISIS 473547, ISIS 473567, ISIS 473589, ISIS 473630, ISIS 484559, ISIS 484713, ISIS 490103, ISIS 490196, ISIS 490208, ISIS 513419, 10 ISIS 513454, ISIS 513455, ISIS 513456, ISIS 513457, ISIS 513487, ISIS 513508, ISIS 515640, ISIS 515641, ISIS 515642, ISIS 515648, ISIS 515655, ISIS 515657, ISIS 516045, ISIS 516046, ISIS 516047, ISIS 516048, ISIS 516051, ISIS 516052, ISIS 516055, ISIS 516056, ISIS 516059, ISIS 516061, ISIS 516062, or ISIS 516063. One group of mice was injected subcutaneously twice a week for 3 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

15 *Protein Analysis*

Plasma protein levels of Factor VII were estimated using a Zymutest FVII ELISA kit (Hyphen Bio-Med cat# ARK036A). Results are presented as percent inhibition of Factor VII, relative to control. As shown in Table 18, several antisense oligonucleotides achieved reduction of human Factor VII relative to the PBS control.

Table 18

Percent inhibition of Factor VII plasma protein levels in transgenic mice

ISIS No	% inhibition
407935	80
416472	49
416549	29
422087	12
422096	21
473137	57
473244	67
473326	42
473327	100
473359	0
473392	22
473393	32
473547	73
473567	77

473589	96
473630	75
484559	75
484713	56
490103	0
490196	74
490208	90
513419	90
513454	83
513455	91
513456	81
513457	12
513487	74
513508	77
515640	83
515641	87
515642	23
515648	32
515655	79
515657	81
516045	52
516046	79
516047	65
516048	79
516051	84
516052	72
516055	70
516056	0
516059	39
516061	64
516062	96
516063	24

Example 18: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Antisense oligonucleotides exhibiting *in vitro* inhibition of Factor VII mRNA were selected and tested at various doses in Hep3B cells. Also tested was ISIS 407939, a 5-10-5 MOE gapmer targeting human Factor VII, which was described in an earlier publication (WO 2009/061851).

Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.074 μ M, 0.222 μ M, 0.667 μ M, 2.000 μ M, and 6.000 μ M concentrations of antisense oligonucleotide, as specified in Table 19. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human Factor VII primer probe

set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 19. As illustrated in Table 19, Factor VII mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. Many of the newly designed antisense oligonucleotides provided in Table 19 achieved an IC₅₀ of less than 2.0 μ M and, therefore, are more potent than ISIS 407939.

Table 19

Dose-dependent antisense inhibition (%) of human Factor VII in Hep3B cells using electroporation

ISIS No	0.074 μ M	0.222 μ M	0.667 μ M	2.000 μ M	6.000 μ M	IC ₅₀ (μ M)
407939	0	9	21	58	76	2.0
515636	14	32	50	62	81	0.7
515639	10	24	41	61	67	1.3
515640	4	16	35	52	63	2.0
515641	0	21	27	55	66	1.9
515642	3	13	36	44	66	2.2
515648	8	10	10	5	16	>6.0
515653	9	35	26	55	71	1.5
515655	0	0	6	13	42	>6.0
515657	0	13	17	38	51	6.0
516045	0	6	15	19	40	>6.0
516046	0	7	32	48	69	2.1
516047	12	27	41	50	63	1.8
516051	9	8	34	52	66	2.0
516052	17	42	27	53	75	1.2
516053	9	7	28	63	77	1.3
516055	0	3	27	54	75	2.0
516056	0	4	14	52	66	2.6
516057	0	34	33	51	70	1.6
516058	13	12	25	47	74	2.0
516059	4	15	36	47	68	1.9
516060	0	1	39	29	63	3.2
516061	0	0	24	0	3	<6.0
516062	0	20	43	65	78	1.0
516063	0	8	10	37	61	3.8
516064	0	3	13	45	69	2.7
516065	0	14	38	63	76	1.3
516066	0	3	30	55	75	1.7

Example 19: Modified antisense oligonucleotides comprising cEt and MOE modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. ISIS 472998, ISIS 515652, ISIS 515653, ISIS 515654, 5 ISIS 515655, ISIS 515656, and ISIS 515657, described in the Examples above were also included in the screen.

The newly designed modified antisense oligonucleotides and their motifs are described in Table 20. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The 'Sugar Chemistry' column provides the sugar modifications 10 throughout each oligonucleotide: 'd' indicates a 2'-deoxynucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'e' indicates a 2'-O-methoxyethyl nucleoside. The 'Sequence' column provides the nucleobase sequence for each SEQ ID NO.

Each oligonucleotide listed in Table 20 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 15 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. 'n/a' indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

20 Activity of newly designed oligonucleotides was compared to ISIS 407939. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII mRNA levels were 25 adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

Table 20

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	% inhibition	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Sugar Chemistry
15263	15276	TGTGCAGCCCGGCA	74	472998	85	2320	2333	kkddddddddddkk
14128	14143	TCCACTGTCCCCCTTG	50	515652	63	1185	1200	eeddddddddddkkk
15195	15210	ATGCATGGTGATGCTT	71	515653	67	2252	2267	eeddddddddddkkk
14708	14723	GAGGCCAGCAGATCAC	86	515654	78	1765	1780	eeddddddddddkkk
15164	15179	TGAAGATGATAATGGA	61	515655	41	2221	2236	eeddddddddddkkk

14713	14728	AGCCTGAGGCCAGCAG	87	515656	74	1770	1785	eeeddddddddkkk
15262	15277	CTGTGCAGCCCGGCAC	73	515657	49	2319	2334	eeeddddddddkkk
n/a	n/a	TGGATATTCAACTGTGG	267	529265	52	551	567	eekdddddddkkeke
1381	1397	GCCTGGATGCTGGTTTC	268	529332	82	n/a	n/a	eekdddddddkkeke
1382	1398	TGCCTGGATGCTGGTTT	269	529334	78	n/a	n/a	eekdddddddkkeke
1383	1399	GTGCCTGGATGCTGGTT	213	529186	85	n/a	n/a	eekdddddddkkeke
1383	1399	GTGCCTGGATGCTGGTT	213	529223	81	n/a	n/a	eekdddddddkkke
1384	1399	GTGCCTGGATGCTGGT	270	529129	75	n/a	n/a	eeeddddddddkkk
1384	1399	GTGCCTGGATGCTGGT	270	529149	82	n/a	n/a	kkkdddddddeee
1384	1400	TGTGCCTGGATGCTGGT	214	529177	77	n/a	n/a	eekdddddddkkeke
1384	1400	TGTGCCTGGATGCTGGT	214	529214	78	n/a	n/a	eekdddddddkkke
1386	1402	CCTGTGCCTGGATGCTG	271	529178	79	n/a	n/a	eekdddddddkkeke
1386	1402	CCTGTGCCTGGATGCTG	271	529215	82	n/a	n/a	eekdddddddkkke
1387	1403	TCCTGTGCCTGGATGCT	272	529179	71	n/a	n/a	eekdddddddkkeke
1387	1403	TCCTGTGCCTGGATGCT	272	529216	77	n/a	n/a	eekdddddddkkke
1388	1404	CTCCTGTGCCTGGATGC	273	529193	69	n/a	n/a	eekdddddddkkeke
1388	1404	CTCCTGTGCCTGGATGC	273	529230	70	n/a	n/a	eekdddddddkkke
1389	1404	CTCCTGTGCCTGGATG	274	529136	48	n/a	n/a	eeeddddddddkkk
1389	1404	CTCCTGTGCCTGGATG	274	529156	68	n/a	n/a	kkkdddddddeee
2229	2245	CAGTCCCTGCTCACCTC	275	529194	44	n/a	n/a	eekdddddddkkeke
2229	2245	CAGTCCCTGCTCACCTC	275	529231	56	n/a	n/a	eekdddddddkkke
2230	2245	CAGTCCCTGCTCACCT	276	529137	34	n/a	n/a	eeeddddddddkkk
2230	2245	CAGTCCCTGCTCACCT	276	529157	79	n/a	n/a	kkkdddddddeee
2235	2251	CAGTGGCAGTCCCTGCT	277	529336	57	n/a	n/a	eekdddddddkkeke
2237	2253	ACCAGTGGCAGTCCCTG	278	529338	73	n/a	n/a	eekdddddddkkeke
2248	2264	CCCAGGACAAAACCAGT	279	529195	55	n/a	n/a	eekdddddddkkeke
2248	2264	CCCAGGACAAAACCAGT	279	529232	68	n/a	n/a	eekdddddddkkke
2272	2288	AGGTGATGTTGGCCCCC	280	529340	65	n/a	n/a	eekdddddddkkeke
2347	2363	AGCAGGGAACACCCTCC	281	529342	69	n/a	n/a	eekdddddddkkeke
2367	2382	GAGCGGTCACTTCCTC	282	529812	69	n/a	n/a	kdddddddkkeee
2367	2382	GAGCGGTCACTTCCTC	282	529831	62	n/a	n/a	kdddddddkdkee
2368	2383	GGAGCGGTCACTTCCT	283	529733	64	n/a	n/a	keddddddddkkeke
2368	2383	GGAGCGGTCACTTCCT	283	529753	52	n/a	n/a	ekdddddddkkeke
2368	2383	GGAGCGGTCACTTCCT	283	529773	57	n/a	n/a	keddddddddkdke
2368	2383	GGAGCGGTCACTTCCT	283	529793	36	n/a	n/a	ekdddddddkdke
2368	2384	TGGAGCGGTCACTTCCT	284	529862	48	n/a	n/a	kdeddddddddkdke
2368	2384	TGGAGCGGTCACTTCCT	284	529882	35	n/a	n/a	edkdddddddkdke
2368	2384	TGGAGCGGTCACTTCCT	284	529902	44	n/a	n/a	kdddkdddkdddkke
2369	2384	TGGAGCGGTCACTTCC	26	529559	71	n/a	n/a	eekdddddddkke
2369	2384	TGGAGCGGTCACTTCC	26	529584	57	n/a	n/a	keeddddddddkke
2369	2384	TGGAGCGGTCACTTCC	26	529609	58	n/a	n/a	edkdddddddkke
2369	2384	TGGAGCGGTCACTTCC	26	529634	49	n/a	n/a	kdeddddddddkke
2369	2384	TGGAGCGGTCACTTCC	26	529659	52	n/a	n/a	kddkdddddddkke

2369	2384	TGGAGCGGTCACTTCC	26	529684	48	n/a	n/a	kddeddddddddkke
2369	2384	TGGAGCGGTCACTTCC	26	529709	61	n/a	n/a	eddkdddddddkke
2369	2384	TGGAGCGGTCACTTCC	26	529922	52	n/a	n/a	eeeddddddddkke
2480	2496	AGCTCTCAAGAACTGAG	285	529344	50	n/a	n/a	eekdddddddkkeke
2489	2504	ATCAGAAAAGCTCTCA	286	529138	32	n/a	n/a	eeddddddddkkk
2489	2504	ATCAGAAAAGCTCTCA	286	529158	75	n/a	n/a	kkkdddddddeee
2490	2506	GCATCAGAAAAGCTCTC	215	529184	75	n/a	n/a	eekdddddddkkeke
2490	2506	GCATCAGAAAAGCTCTC	215	529221	78	n/a	n/a	eekdddddddkkke
2491	2506	GCATCAGAAAAGCTCT	287	529127	67	n/a	n/a	eeddddddddkkk
2491	2506	GCATCAGAAAAGCTCT	287	529147	79	n/a	n/a	kkkdddddddeee
2491	2507	TGCATCAGAAAAGCTCT	288	529346	58	n/a	n/a	eekdddddddkkeke
2497	2513	CAGATTTGCATCAGAAA	289	529348	65	n/a	n/a	eekdddddddkkeke
2498	2514	GCAGATTTGCATCAGAA	290	529350	77	n/a	n/a	eekdddddddkkeke
4715	4730	GAGGCTGAGGATGCAG	291	529813	20	n/a	n/a	kdddddddkkeke
4715	4730	GAGGCTGAGGATGCAG	291	529832	47	n/a	n/a	kdddddddkdke
4716	4731	GGAGGCTGAGGATGCA	292	529734	63	n/a	n/a	keddddddddkkeke
4716	4731	GGAGGCTGAGGATGCA	292	529754	58	n/a	n/a	ekdddddddkkeke
4716	4731	GGAGGCTGAGGATGCA	292	529774	49	n/a	n/a	kedddddddkdke
4716	4731	GGAGGCTGAGGATGCA	292	529794	51	n/a	n/a	ekddddddkdke
4716	4732	AGGAGGCTGAGGATGCA	293	529863	64	n/a	n/a	kdeddddddkdke
4716	4732	AGGAGGCTGAGGATGCA	293	529883	78	n/a	n/a	edkdddddddkdke
4716	4732	AGGAGGCTGAGGATGCA	293	529903	36	n/a	n/a	kdddkdddkdddkke
4717	4732	AGGAGGCTGAGGATGC	27	529560	71	n/a	n/a	eekdddddddkke
4717	4732	AGGAGGCTGAGGATGC	27	529585	70	n/a	n/a	keeddddddddkke
4717	4732	AGGAGGCTGAGGATGC	27	529610	66	n/a	n/a	edkdddddddkke
4717	4732	AGGAGGCTGAGGATGC	27	529635	45	n/a	n/a	kdeddddddddkke
4717	4732	AGGAGGCTGAGGATGC	27	529660	53	n/a	n/a	kddkdddddddkke
4717	4732	AGGAGGCTGAGGATGC	27	529685	42	n/a	n/a	kddeddddddkke
4717	4732	AGGAGGCTGAGGATGC	27	529710	60	n/a	n/a	eddkdddddddkke
4717	4732	AGGAGGCTGAGGATGC	27	529923	63	n/a	n/a	eeeddddddddkke
4726	4742	CTGGTTTGGAAGGAGGC	294	529196	74	n/a	n/a	eekdddddddkkeke
4726	4742	CTGGTTTGGAAGGAGGC	294	529233	80	n/a	n/a	eekdddddddkkke
4727	4742	CTGGTTTGGAAGGAGG	295	529139	75	n/a	n/a	eeddddddddkkk
4727	4742	CTGGTTTGGAAGGAGG	295	529159	62	n/a	n/a	kkkdddddddeee
4728	4744	GTCTGGTTTGGAAGGAG	296	529352	74	n/a	n/a	eekdddddddkkeke
4816	4832	GCGCTACTGGGCCACGT	297	529354	67	n/a	n/a	eekdddddddkkeke
4848	4864	TTACTGAGCGCGGAAGA	298	529197	43	n/a	n/a	eekdddddddkkeke
4848	4864	TTACTGAGCGCGGAAGA	298	529234	58	n/a	n/a	eekdddddddkkke
4849	4864	TTACTGAGCGCGGAAG	299	529140	29	n/a	n/a	eeddddddddkkk
4849	4864	TTACTGAGCGCGGAAG	299	529160	59	n/a	n/a	kkkdddddddeee
4850	4866	GGTTACTGAGCGCGGAA	216	529180	80	n/a	n/a	eekdddddddkkeke
4850	4866	GGTTACTGAGCGCGGAA	216	529217	79	n/a	n/a	eekdddddddkkke
4869	4884	GCAGGAGCGGCCTAAA	300	529814	51	n/a	n/a	kdddddddkkeke

4869	4884	GCAGGAGCGGCCTAAA	300	529833	52	n/a	n/a	kddddddddddkdkke
4870	4885	TGCAGGAGCGGCCTAA	301	529735	43	n/a	n/a	kedddddddddddkeke
4870	4885	TGCAGGAGCGGCCTAA	301	529755	60	n/a	n/a	ekddddddddddkeke
4870	4885	TGCAGGAGCGGCCTAA	301	529775	38	n/a	n/a	kedddddddddddkdkke
4870	4885	TGCAGGAGCGGCCTAA	301	529795	58	n/a	n/a	ekddddddddddkdkke
4870	4886	CTGCAGGAGCGGCCTAA	302	529864	41	n/a	n/a	kdedddddddddkdkke
4870	4886	CTGCAGGAGCGGCCTAA	302	529884	48	n/a	n/a	edkddddddddddkdkke
4870	4886	CTGCAGGAGCGGCCTAA	302	529904	44	n/a	n/a	kdddkdddkddkdddkke
4870	4886	CTGCAGGAGCGGCCTAA	302	529934	61	n/a	n/a	eekddddddddddkeke
4871	4887	TCTGCAGGAGCGGCCTA	303	529356	71	n/a	n/a	eekddddddddddkeke
4871	4886	CTGCAGGAGCGGCCTA	28	529561	75	n/a	n/a	eekddddddddddkdkke
4871	4886	CTGCAGGAGCGGCCTA	28	529586	65	n/a	n/a	keedddddddddddkdkke
4871	4886	CTGCAGGAGCGGCCTA	28	529611	54	n/a	n/a	edkddddddddddkdkke
4871	4886	CTGCAGGAGCGGCCTA	28	529636	39	n/a	n/a	kdedddddddddddkdkke
4871	4886	CTGCAGGAGCGGCCTA	28	529661	67	n/a	n/a	kddkddddddddddkdkke
4871	4886	CTGCAGGAGCGGCCTA	28	529686	66	n/a	n/a	kddedddddddddddkdkke
4871	4886	CTGCAGGAGCGGCCTA	28	529711	60	n/a	n/a	eddkddddddddddkdkke
4871	4886	CTGCAGGAGCGGCCTA	28	529924	62	n/a	n/a	eeeedddddddddddkdkke
4872	4888	TTCTGCAGGAGCGGCCT	304	529358	82	n/a	n/a	eekddddddddddkeke
4873	4889	GTTCTGCAGGAGCGGCC	217	529181	79	n/a	n/a	eekddddddddddkeke
4873	4889	GTTCTGCAGGAGCGGCC	217	529218	73	n/a	n/a	eekddddddddddkdkke
4874	4890	AGTTCTGCAGGAGCGGC	218	529182	85	n/a	n/a	eekddddddddddkeke
4874	4890	AGTTCTGCAGGAGCGGC	218	529219	84	n/a	n/a	eekddddddddddkdkke
4875	4891	GAGTTCTGCAGGAGCGG	305	529360	84	n/a	n/a	eekddddddddddkeke
4876	4892	CGAGTTCTGCAGGAGCG	306	529362	87	n/a	n/a	eekddddddddddkeke
4877	4893	CCGAGTTCTGCAGGAGC	307	529364	81	n/a	n/a	eekddddddddddkeke
4921	4937	AGGACGAGGCCTCAGGT	308	529366	77	n/a	n/a	eekddddddddddkeke
4940	4956	AGGGACGACCTTTGCTG	309	529198	28	n/a	n/a	eekddddddddddkeke
4940	4956	AGGGACGACCTTTGCTG	309	529235	8	n/a	n/a	eekddddddddddkdkke
4941	4956	AGGGACGACCTTTGCT	310	529141	34	n/a	n/a	eeedddddddddddkkk
4941	4956	AGGGACGACCTTTGCT	310	529161	66	n/a	n/a	kkkdddddddddddeee
5127	5143	GTGGGACACGCGGTG	311	529368	27	n/a	n/a	eekddddddddddkeke
5128	5144	TGTGGGACACGCGGT	312	529370	44	n/a	n/a	eekddddddddddkeke
5131	5147	AGCTGTGGGACACGCG	313	529372	61	n/a	n/a	eekddddddddddkeke
5132	5148	GAGCTGTGGGACACGCG	314	529374	71	n/a	n/a	eekddddddddddkeke
5133	5149	TGAGCTGTGGGACACAC	315	529376	63	n/a	n/a	eekddddddddddkeke
5373	5389	GACCTCGCGCGGATCCT	316	529378	68	n/a	n/a	eekddddddddddkeke
5511	5527	CCGAGGCGCGGCCCTG	317	529380	79	n/a	n/a	eekddddddddddkeke
5512	5528	TCCGAGGCGCGGCCCT	318	529382	77	n/a	n/a	eekddddddddddkeke
5556	5572	CGTCTCCGCGGCCAGA	319	529384	75	n/a	n/a	eekddddddddddkeke
5601	5617	ACAGCCGCCCGCGGAAA	320	529386	40	n/a	n/a	eekddddddddddkeke
6075	6091	CCGGCCGCAGCTCCTCC	321	529240	73	180	196	eekddddddddddkeke
6076	6092	CCCGGCCGCAGCTCCTC	322	529241	67	181	197	eekddddddddddkeke

6100	6116	TCCTTGCACTCCCTCTC	323	529242	42	205	221	eekddddddddddkeke
6132	6148	TCTCCCGGGCCTCCTCG	324	529243	60	237	253	eekddddddddddkeke
6401	6417	TGATGTGAAAACCGGCA	325	529388	65	n/a	n/a	eekddddddddddkeke
6409	6424	TATTTTCTGATGTGAA	326	529815	37	n/a	n/a	kddddddddddkekee
6409	6424	TATTTTCTGATGTGAA	326	529834	44	n/a	n/a	kddddddddddkdkkee
6410	6425	GTATTTTCTGATGTGA	327	529736	47	n/a	n/a	kedddddddddddkeke
6410	6425	GTATTTTCTGATGTGA	327	529756	78	n/a	n/a	ekddddddddddkeke
6410	6425	GTATTTTCTGATGTGA	327	529776	37	n/a	n/a	kedddddddddddkdke
6410	6425	GTATTTTCTGATGTGA	327	529796	71	n/a	n/a	ekddddddddddkdke
6410	6426	CGTATTTTCTGATGTGA	328	529865	70	n/a	n/a	kdedddddddddddkdke
6410	6426	CGTATTTTCTGATGTGA	328	529885	59	n/a	n/a	edkddddddddddkdke
6410	6426	CGTATTTTCTGATGTGA	328	529905	54	n/a	n/a	kdddkdddkdddkddke
6410	6426	CGTATTTTCTGATGTGA	328	529935	70	n/a	n/a	eekddddddddddkeke
6411	6426	CGTATTTTCTGATGTG	29	529562	87	n/a	n/a	eekddddddddddkke
6411	6426	CGTATTTTCTGATGTG	29	529587	68	n/a	n/a	keedddddddddddkke
6411	6426	CGTATTTTCTGATGTG	29	529612	67	n/a	n/a	edkddddddddddkke
6411	6426	CGTATTTTCTGATGTG	29	529637	64	n/a	n/a	kdedddddddddddkke
6411	6426	CGTATTTTCTGATGTG	29	529662	62	n/a	n/a	kddkddddddddddkke
6411	6426	CGTATTTTCTGATGTG	29	529687	63	n/a	n/a	kddedddddddddddkke
6411	6426	CGTATTTTCTGATGTG	29	529712	61	n/a	n/a	eddkddddddddddkke
6411	6426	CGTATTTTCTGATGTG	29	529925	61	n/a	n/a	eeeedddddddddkke
6640	6655	GGTGACCCGTGAGCGT	329	529816	77	n/a	n/a	kddddddddddkekee
6640	6655	GGTGACCCGTGAGCGT	329	529835	80	n/a	n/a	kddddddddddkdkkee
6641	6656	AGGTGACCCGTGAGCG	330	529737	82	n/a	n/a	kedddddddddddkeke
6641	6656	AGGTGACCCGTGAGCG	330	529757	83	n/a	n/a	ekddddddddddkeke
6641	6656	AGGTGACCCGTGAGCG	330	529777	68	n/a	n/a	kedddddddddddkdke
6641	6656	AGGTGACCCGTGAGCG	330	529797	77	n/a	n/a	ekddddddddddkdke
6641	6657	GAGGTGACCCGTGAGCG	331	529866	15	n/a	n/a	kdedddddddddddkdke
6641	6657	GAGGTGACCCGTGAGCG	331	529886	71	n/a	n/a	edkddddddddddkdke
6641	6657	GAGGTGACCCGTGAGCG	331	529906	63	n/a	n/a	kdddkdddkdddkddke
6641	6657	GAGGTGACCCGTGAGCG	331	529936	78	n/a	n/a	eekddddddddddkeke
6642	6657	GAGGTGACCCGTGAGC	30	529563	89	n/a	n/a	eekddddddddddkke
6642	6657	GAGGTGACCCGTGAGC	30	529588	84	n/a	n/a	keedddddddddddkke
6642	6657	GAGGTGACCCGTGAGC	30	529613	80	n/a	n/a	edkddddddddddkke
6642	6657	GAGGTGACCCGTGAGC	30	529638	48	n/a	n/a	kdedddddddddddkke
6642	6657	GAGGTGACCCGTGAGC	30	529663	85	n/a	n/a	kddkddddddddddkke
6642	6657	GAGGTGACCCGTGAGC	30	529688	42	n/a	n/a	kddedddddddddddkke
6642	6657	GAGGTGACCCGTGAGC	30	529713	81	n/a	n/a	eddkddddddddddkke
6642	6657	GAGGTGACCCGTGAGC	30	529926	67	n/a	n/a	eeeedddddddddkke
8548	8564	GGCATGACCATCCTCAA	332	529390	53	n/a	n/a	eekddddddddddkeke
8553	8569	TGCTAGGCATGACCATC	333	529392	63	n/a	n/a	eekddddddddddkeke
8606	8622	TGCTGTGAGAATACAAC	334	529394	58	n/a	n/a	eekddddddddddkeke
8608	8624	GGTGCTGTGAGAATACA	335	529396	56	n/a	n/a	eekddddddddddkeke

8642	8658	AGAAACTGTTGGCCACC	336	529398	62	n/a	n/a	eekddddddddddkeke
8643	8659	AAGAAACTGTTGGCCAC	337	529400	44	n/a	n/a	eekddddddddddkeke
8654	8670	AGTGATTGCTGAAGAAA	338	529402	39	n/a	n/a	eekddddddddddkeke
8707	8723	GCAGTAGCAGATGCAAA	339	529404	46	n/a	n/a	eekddddddddddkeke
8860	8876	ATGGGTGACCACACATT	340	529406	63	n/a	n/a	eekddddddddddkeke
9168	9184	GGCACACTGGTCCCCAT	341	529244	58	302	318	eekddddddddddkeke
9169	9185	AGGCACACTGGTCCCCA	342	529245	68	303	319	eekddddddddddkeke
9171	9187	TGAGGCACACTGGTCCC	343	529246	60	305	321	eekddddddddddkeke
9199	9215	TGCAGGAGCCCCATTCT	344	529247	36	333	349	eekddddddddddkeke
9212	9228	TGGAGCTGGTCTTGCA	345	529248	43	346	362	eekddddddddddkeke
9217	9233	AGGACTGGAGCTGGTCC	346	529249	23	351	367	eekddddddddddkeke
9224	9240	CAGATATAGGACTGGAG	347	529250	69	358	374	eekddddddddddkeke
9226	9242	AGCAGATATAGGACTGG	348	529251	15	360	376	eekddddddddddkeke
9258	9274	ACAGTTCGGGCCCTCGA	349	529252	44	392	408	eekddddddddddkeke
9261	9277	CTCACAGTTCCGGCCCT	350	529253	42	395	411	eekddddddddddkeke
9356	9372	AGGTTGTGCACCCTGCA	351	529408	67	n/a	n/a	eekddddddddddkeke
9358	9374	CCAGGTTGTGCACCCTG	352	529410	19	n/a	n/a	eekddddddddddkeke
9452	9468	CCTGTCAAAGACCTCAG	353	529412	57	n/a	n/a	eekddddddddddkeke
9598	9614	GAAGTTTACCAAGCGGT	354	529414	80	n/a	n/a	eekddddddddddkeke
9939	9955	AACCTCTGGACACCGGG	355	529416	85	n/a	n/a	eekddddddddddkeke
9958	9974	TGTGATTGAGCCCTGAT	356	529418	70	n/a	n/a	eekddddddddddkeke
10067	10083	TGGTCTAGCTGACAATG	357	529420	78	n/a	n/a	eekddddddddddkeke
10074	10090	TGCTGGATGGTCTAGCT	358	529422	19	n/a	n/a	eekddddddddddkeke
10334	10350	GGCATTCTGGACCCAGA	359	529424	48	n/a	n/a	eekddddddddddkeke
10383	10399	AGGATGCACACCAGGGC	360	529426	66	n/a	n/a	eekddddddddddkeke
10384	10400	CAGGATGCACACCAGGG	361	529428	59	n/a	n/a	eekddddddddddkeke
10417	10433	AAGTCCAGGACTCCGGC	362	529430	83	n/a	n/a	eekddddddddddkeke
10669	10685	CCGAGCAGCTGATGGGA	363	529432	84	n/a	n/a	eekddddddddddkeke
10677	10693	CCACTCTTCCGAGCAGC	364	529199	71	n/a	n/a	eekddddddddddkeke
10677	10693	CCACTCTTCCGAGCAGC	364	529236	76	n/a	n/a	eekddddddddddkkke
10678	10693	CCACTCTTCCGAGCAG	365	529142	64	n/a	n/a	eedddddddddddkkk
10678	10693	CCACTCTTCCGAGCAG	365	529162	60	n/a	n/a	kkkdddddddddeee
10983	10999	ATCAGCTGGTCATCCTT	366	529254	46	418	434	eekddddddddddkeke
10986	11002	CAGATCAGCTGGTCATC	367	529255	52	421	437	eekddddddddddkeke
11013	11029	TGCTCACAGCCGCCGTT	368	529256	57	448	464	eekddddddddddkeke
11014	11030	CTGCTCACAGCCGCCGT	369	529257	55	449	465	eekddddddddddkeke
11015	11031	ACTGCTCACAGCCGCCG	370	529258	3	450	466	eekddddddddddkeke
11016	11032	TACTGCTCACAGCCGCC	371	529259	71	451	467	eekddddddddddkeke
11018	11034	AGTACTGCTCACAGCCG	372	529260	72	453	469	eekddddddddddkeke
11021	11037	TGCAGTACTGCTCACAG	373	529261	56	456	472	eekddddddddddkeke
11025	11041	TACTGCAGTACTGCTC	374	529262	56	460	476	eekddddddddddkeke
11028	11044	TGGTCACTGCAGTACTG	375	529263	59	463	479	eekddddddddddkeke
11089	11105	CACCCCGTCTGCCAGCA	376	529264	49	524	540	eekddddddddddkeke

11142	11158	TGGTGTCTATCTGGGACT	377	529434	83	n/a	n/a	eekddddddddddkeke
11162	11178	ATCCGTAGTGGGACAGG	378	529436	80	n/a	n/a	eekddddddddddkeke
11279	11295	TGTCGCTCTGGCCTGTG	379	529438	79	n/a	n/a	eekddddddddddkeke
11281	11297	ACTGTCGCTCTGGCCTG	380	529440	87	n/a	n/a	eekddddddddddkeke
11284	11300	GTCAGTGTCTGCTCTGGC	381	529442	68	n/a	n/a	eekddddddddddkeke
11401	11417	AGGTCCTGCGAGTGGGA	382	529443	72	n/a	n/a	eekddddddddddkeke
11403	11419	GGAGGTCCTGCGAGTGG	383	529444	68	n/a	n/a	eekddddddddddkeke
11454	11470	AGCAGTCAGTACAGACA	384	529445	85	n/a	n/a	eekddddddddddkeke
11543	11559	TGGAAGTGCAGCCCATT	385	529446	72	n/a	n/a	eekddddddddddkeke
11544	11560	TTGGAAGTGCAGCCCATT	386	529447	60	n/a	n/a	eekddddddddddkeke
11836	11852	TGGTCAGGGCTGGTTTT	387	529448	77	n/a	n/a	eekddddddddddkeke
11837	11852	TGGTCAGGGCTGGTTTT	388	529807	78	n/a	n/a	kddddddddddkekee
11837	11852	TGGTCAGGGCTGGTTTT	388	529826	61	n/a	n/a	kddddddddddkdkke
11838	11854	AATGGTCAGGGCTGGTT	389	529449	81	n/a	n/a	eekddddddddddkeke
11838	11853	ATGGTCAGGGCTGGTT	390	529728	75	n/a	n/a	kedddddddddddkeke
11838	11853	ATGGTCAGGGCTGGTT	390	529748	80	n/a	n/a	ekddddddddddkeke
11838	11853	ATGGTCAGGGCTGGTT	390	529768	68	n/a	n/a	kedddddddddddkdkke
11838	11853	ATGGTCAGGGCTGGTT	390	529788	74	n/a	n/a	ekddddddddddkdkke
11838	11854	AATGGTCAGGGCTGGTT	389	529857	67	n/a	n/a	kdedddddddddddkdkke
11838	11854	AATGGTCAGGGCTGGTT	389	529877	77	n/a	n/a	edkddddddddddkdkke
11838	11854	AATGGTCAGGGCTGGTT	389	529897	26	n/a	n/a	kdddkdddkddkdddkke
11839	11855	CAATGGTCAGGGCTGGT	391	529200	78	n/a	n/a	eekddddddddddkeke
11839	11855	CAATGGTCAGGGCTGGT	391	529237	84	n/a	n/a	eekddddddddddkkke
11839	11854	AATGGTCAGGGCTGGT	34	529564	90	n/a	n/a	eekddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	529589	86	n/a	n/a	keedddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	529614	82	n/a	n/a	edkddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	529639	80	n/a	n/a	kdedddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	529664	69	n/a	n/a	kddkddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	529689	71	n/a	n/a	kddedddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	529714	73	n/a	n/a	eddkddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	529917	73	n/a	n/a	eeedddddddddddkke
11840	11855	CAATGGTCAGGGCTGG	392	529143	68	n/a	n/a	eeedddddddddddkkk
11840	11855	CAATGGTCAGGGCTGG	392	529163	50	n/a	n/a	kkkdddddddddddeee
11840	11856	ACAATGGTCAGGGCTGG	393	529201	76	n/a	n/a	eekddddddddddkeke
11840	11856	ACAATGGTCAGGGCTGG	393	529238	72	n/a	n/a	eekddddddddddkkke
11841	11856	ACAATGGTCAGGGCTG	394	529144	57	n/a	n/a	eeedddddddddddkkk
11841	11856	ACAATGGTCAGGGCTG	394	529164	71	n/a	n/a	kkkdddddddddddeee
11845	11861	AGGAGACAATGGTCAGG	395	529450	91	n/a	n/a	eekddddddddddkeke
11846	11862	GAGGAGACAATGGTCAG	396	529451	85	n/a	n/a	eekddddddddddkeke
12097	12113	TAGGTATTTTCCACAT	397	529266	63	567	583	eekddddddddddkeke
12125	12140	GTTTGCTGGCATTCT	398	529806	52	595	610	kddddddddddkekee
12125	12140	GTTTGCTGGCATTCT	398	529825	44	595	610	kddddddddddkdkke
12126	12142	GGGTTTGCTGGCATTCT	399	529267	56	596	612	eekddddddddddkeke

12126	12141	GGTTTGCTGGCATTTC	400	529727	67	596	611	kedddddddddddkeke
12126	12141	GGTTTGCTGGCATTTC	400	529747	63	596	611	ekddddddddddkeke
12126	12141	GGTTTGCTGGCATTTC	400	529767	67	596	611	kedddddddddddkdke
12126	12141	GGTTTGCTGGCATTTC	400	529787	68	596	611	ekddddddddddkdke
12126	12142	GGGTTTGCTGGCATTTC	399	529856	42	596	612	kdedddddddddddkdke
12126	12142	GGGTTTGCTGGCATTTC	399	529876	36	596	612	edkddddddddddkdke
12126	12142	GGGTTTGCTGGCATTTC	399	529896	56	596	612	kdddkdddkdddkddke
12127	12142	GGGTTTGCTGGCATTT	248	529546	65	597	612	eedddddddddddkke
12127	12142	GGGTTTGCTGGCATTT	248	529571	80	597	612	keedddddddddddkke
12127	12142	GGGTTTGCTGGCATTT	248	529596	43	597	612	edkddddddddddkke
12127	12142	GGGTTTGCTGGCATTT	248	529621	38	597	612	kdedddddddddddkke
12127	12142	GGGTTTGCTGGCATTT	248	529646	68	597	612	kddkddddddddddkke
12127	12142	GGGTTTGCTGGCATTT	248	529671	50	597	612	kddedddddddddkke
12127	12142	GGGTTTGCTGGCATTT	248	529696	53	597	612	eddkddddddddddkke
12127	12142	GGGTTTGCTGGCATTT	248	529916	22	597	612	eeeedddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529547	86	611	626	eedddddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529572	75	611	626	keedddddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529597	58	611	626	edkddddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529622	58	611	626	kdedddddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529647	18	611	626	kddkddddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529672	23	611	626	kddedddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529697	28	611	626	eddkddddddddddkke
12141	12156	ACAATTCGGCCTTGGG	37	529928	36	611	626	eeeedddddddddkke
12302	12318	TGACTTGAGCCTGGTG	401	529452	63	n/a	n/a	eedddddddddddkeke
12404	12420	TTCTCTGCACAGGGTAG	402	529453	73	n/a	n/a	eedddddddddddkeke
12504	12520	TGATCCAATGCTCCTGA	403	529454	82	n/a	n/a	eedddddddddddkeke
12505	12521	TTGATCCAATGCTCCTG	404	529455	84	n/a	n/a	eedddddddddddkeke
12506	12522	TTTGATCCAATGCTCCT	405	529202	61	n/a	n/a	eedddddddddddkeke
12506	12522	TTTGATCCAATGCTCCT	405	529239	59	n/a	n/a	eedddddddddddkke
12507	12522	TTTGATCCAATGCTCC	406	529145	54	n/a	n/a	eeeddddddddddkkk
12507	12522	TTTGATCCAATGCTCC	406	529165	77	n/a	n/a	kkkdddddddddeee
12509	12525	AGCTTTGATCCAATGCT	407	529456	69	n/a	n/a	eedddddddddddkeke
12511	12527	ATAGCTTTGATCCAATG	408	529457	81	n/a	n/a	eedddddddddddkeke
12512	12528	CATAGCTTTGATCCAAT	409	529458	72	n/a	n/a	eedddddddddddkeke
12519	12535	ATCTTCACATAGCTTTG	410	529459	86	n/a	n/a	eedddddddddddkeke
12552	12568	GTCGCTGTGAGATTCA	411	529460	88	n/a	n/a	eedddddddddddkeke
12627	12642	TCAGACCTGGCTCTGG	412	529817	46	n/a	n/a	kddddddddddkekee
12627	12642	TCAGACCTGGCTCTGG	412	529836	49	n/a	n/a	kddddddddddkdkee
12628	12643	CTCAGACCTGGCTCTG	413	529738	51	n/a	n/a	kedddddddddddkeke
12628	12643	CTCAGACCTGGCTCTG	413	529758	53	n/a	n/a	ekddddddddddkeke
12628	12643	CTCAGACCTGGCTCTG	413	529778	39	n/a	n/a	kedddddddddddkdke
12628	12643	CTCAGACCTGGCTCTG	413	529798	52	n/a	n/a	ekddddddddddkdke
12628	12644	GCTCAGACCTGGCTCTG	414	529867	56	n/a	n/a	kdedddddddddddkdke

12628	12644	GCTCAGACCTGGCTCTG	414	529887	68	n/a	n/a	edkddddddddddkdkke
12628	12644	GCTCAGACCTGGCTCTG	414	529907	28	n/a	n/a	kddddkdddkdddkke
12628	12644	GCTCAGACCTGGCTCTG	414	529938	64	n/a	n/a	eekddddddddddkeke
12629	12644	GCTCAGACCTGGCTCT	38	529565	81	n/a	n/a	eekddddddddddkke
12629	12644	GCTCAGACCTGGCTCT	38	529590	49	n/a	n/a	keedddddddddddkke
12629	12644	GCTCAGACCTGGCTCT	38	529615	65	n/a	n/a	edkddddddddddkke
12629	12644	GCTCAGACCTGGCTCT	38	529640	54	n/a	n/a	kdedddddddddddkke
12629	12644	GCTCAGACCTGGCTCT	38	529665	77	n/a	n/a	kdkddddddddddkke
12629	12644	GCTCAGACCTGGCTCT	38	529690	77	n/a	n/a	kdedddddddddddkke
12629	12644	GCTCAGACCTGGCTCT	38	529715	63	n/a	n/a	eddkddddddddddkke
12629	12644	GCTCAGACCTGGCTCT	38	529927	62	n/a	n/a	eeeedddddddddkke
12631	12647	GCTGCTCAGACCTGGCT	221	529185	66	n/a	n/a	eekddddddddddkeke
12631	12647	GCTGCTCAGACCTGGCT	221	529222	62	n/a	n/a	eekddddddddddkkke
12631	12646	CTGCTCAGACCTGGCT	89	529808	75	n/a	n/a	kddddddddddkekee
12631	12646	CTGCTCAGACCTGGCT	89	529827	67	n/a	n/a	kddddddddddkdkkee
12632	12647	GCTGCTCAGACCTGGC	415	529128	64	n/a	n/a	eedddddddddddkkk
12632	12647	GCTGCTCAGACCTGGC	415	529148	78	n/a	n/a	kkkdddddddddeeee
12632	12648	AGCTGCTCAGACCTGGC	416	529461	87	n/a	n/a	eekddddddddddkeke
12632	12647	GCTGCTCAGACCTGGC	415	529729	71	n/a	n/a	kedddddddddddkeke
12632	12647	GCTGCTCAGACCTGGC	415	529749	83	n/a	n/a	ekddddddddddkeke
12632	12647	GCTGCTCAGACCTGGC	415	529769	63	n/a	n/a	kedddddddddddkdkke
12632	12647	GCTGCTCAGACCTGGC	415	529789	10	n/a	n/a	ekddddddddddkdkke
12632	12647	GCTGCTCAGACCTGGC	415	529800	69	n/a	n/a	kddddddddddkekee
12632	12647	GCTGCTCAGACCTGGC	415	529819	78	n/a	n/a	kddddddddddkdkkee
12632	12648	AGCTGCTCAGACCTGGC	416	529858	60	n/a	n/a	kdedddddddddddkdkke
12632	12648	AGCTGCTCAGACCTGGC	416	529878	75	n/a	n/a	edkddddddddddkdkke
12632	12648	AGCTGCTCAGACCTGGC	416	529898	34	n/a	n/a	kdddkkdddkkdddkke
12633	12648	AGCTGCTCAGACCTGG	39	529566	61	n/a	n/a	eekddddddddddkke
12633	12648	AGCTGCTCAGACCTGG	39	529591	71	n/a	n/a	keedddddddddddkke
12633	12648	AGCTGCTCAGACCTGG	39	529616	71	n/a	n/a	edkddddddddddkke
12633	12648	AGCTGCTCAGACCTGG	39	529641	65	n/a	n/a	kdedddddddddddkke
12633	12648	AGCTGCTCAGACCTGG	39	529666	70	n/a	n/a	kdkddddddddddkke
12633	12648	AGCTGCTCAGACCTGG	39	529691	67	n/a	n/a	kdedddddddddddkke
12633	12648	AGCTGCTCAGACCTGG	39	529716	75	n/a	n/a	eddkddddddddddkke
12633	12648	AGCTGCTCAGACCTGG	39	529721	71	n/a	n/a	kedddddddddddkeke
12633	12648	AGCTGCTCAGACCTGG	39	529741	81	n/a	n/a	ekddddddddddkeke
12633	12648	AGCTGCTCAGACCTGG	39	529761	66	n/a	n/a	kedddddddddddkdkke
12633	12648	AGCTGCTCAGACCTGG	39	529781	65	n/a	n/a	ekddddddddddkdkke
12633	12648	AGCTGCTCAGACCTGG	39	529801	71	n/a	n/a	kddddddddddkekee
12633	12648	AGCTGCTCAGACCTGG	39	529820	74	n/a	n/a	kddddddddddkdkkee
12633	12649	AAGCTGCTCAGACCTGG	417	529850	63	n/a	n/a	kdedddddddddddkdkke
12633	12649	AAGCTGCTCAGACCTGG	417	529870	72	n/a	n/a	edkddddddddddkdkke
12633	12649	AAGCTGCTCAGACCTGG	417	529890	23	n/a	n/a	kdddkkdddkkdddkke

12633	12648	AGCTGCTCAGACCTGG	39	529918	54	n/a	n/a	eeeeddddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529567	75	n/a	n/a	eekdddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529592	80	n/a	n/a	keeddddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529617	65	n/a	n/a	edkdddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529642	62	n/a	n/a	kdeddddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529667	75	n/a	n/a	kddkdddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529692	53	n/a	n/a	kddeddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529717	69	n/a	n/a	eddkdddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529722	74	n/a	n/a	keddddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529742	81	n/a	n/a	ekdddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529762	66	n/a	n/a	keddddddddkke
12634	12649	AAGCTGCTCAGACCTG	262	529782	68	n/a	n/a	ekdddddddkke
12634	12650	AAAGCTGCTCAGACCTG	418	529851	68	n/a	n/a	kdeddddddddkke
12634	12650	AAAGCTGCTCAGACCTG	418	529871	77	n/a	n/a	edkdddddddkke
12634	12650	AAAGCTGCTCAGACCTG	418	529891	36	n/a	n/a	kdddkdddkdddkke
12634	12649	AAGCTGCTCAGACCTG	262	529910	60	n/a	n/a	eeeeddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529568	79	n/a	n/a	eekdddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529593	70	n/a	n/a	keeddddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529618	77	n/a	n/a	edkdddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529643	72	n/a	n/a	kdeddddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529668	73	n/a	n/a	kddkdddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529693	62	n/a	n/a	kddeddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529718	69	n/a	n/a	eddkdddddddkke
12635	12650	AAAGCTGCTCAGACCT	263	529911	66	n/a	n/a	eeeeddddddkke
12693	12709	CTCTGGCATTGCACAAT	419	529462	76	n/a	n/a	eekdddddddkke
12794	12810	TTCACCAACAACAGGAC	420	529268	18	667	683	eekdddddddkke
12802	12818	GAGCTCCATTACCAAC	421	529187	46	675	691	eekdddddddkke
12802	12818	GAGCTCCATTACCAAC	421	529224	48	675	691	eekdddddddkke
12803	12818	GAGCTCCATTACCAA	422	529130	34	676	691	eeeddddddddkk
12803	12818	GAGCTCCATTACCAA	422	529150	51	676	691	kkkdddddddeee
12863	12878	CGAAACAGTGGGCCGC	42	529549	85	736	751	eekdddddddkke
12863	12878	CGAAACAGTGGGCCGC	42	529574	81	736	751	keeddddddddkke
12863	12878	CGAAACAGTGGGCCGC	42	529599	64	736	751	edkdddddddkke
12863	12878	CGAAACAGTGGGCCGC	42	529624	68	736	751	kdeddddddddkke
12863	12878	CGAAACAGTGGGCCGC	42	529649	77	736	751	kddkdddddddkke
12863	12878	CGAAACAGTGGGCCGC	42	529674	65	736	751	kddeddddddkke
12863	12878	CGAAACAGTGGGCCGC	42	529699	63	736	751	eddkdddddddkke
12863	12878	CGAAACAGTGGGCCGC	42	529931	59	736	751	eeeeddddddkke
13739	13754	GCTCGCTGAGGTCGTG	423	529810	80	796	811	kddddddddkekee
13739	13754	GCTCGCTGAGGTCGTG	423	529829	67	796	811	kdddddddddkkee
13740	13756	GTGCTCGCTGAGGTCGT	424	529269	65	797	813	eekdddddddkke
13740	13755	TGCTCGCTGAGGTCGT	425	529731	66	797	812	keddddddddkke
13740	13755	TGCTCGCTGAGGTCGT	425	529751	76	797	812	ekdddddddkke

13740	13755	TGCTCGCTGAGGTCGT	425	529771	73	797	812	keddddddddddkdke
13740	13755	TGCTCGCTGAGGTCGT	425	529791	65	797	812	ekdddddddddkdke
13740	13756	GTGCTCGCTGAGGTCGT	424	529860	73	797	813	kdeddddddddddkdke
13740	13756	GTGCTCGCTGAGGTCGT	424	529880	74	797	813	edkdddddddddkdke
13740	13756	GTGCTCGCTGAGGTCGT	424	529900	62	797	813	kdddkdddddksddkde
13741	13757	CGTGCTCGCTGAGGTCG	480	529270	69	798	814	eekdddddddddkeke
13741	13756	GTGCTCGCTGAGGTCG	44	529550	81	798	813	eekdddddddddkke
13741	13756	GTGCTCGCTGAGGTCG	44	529575	88	798	813	keeddddddddddkke
13741	13756	GTGCTCGCTGAGGTCG	44	529600	78	798	813	edkdddddddddkke
13741	13756	GTGCTCGCTGAGGTCG	44	529625	74	798	813	kdeddddddddddkke
13741	13756	GTGCTCGCTGAGGTCG	44	529650	81	798	813	kddkdddddddddkke
13741	13756	GTGCTCGCTGAGGTCG	44	529675	76	798	813	kddeddddddddddkke
13741	13756	GTGCTCGCTGAGGTCG	44	529700	73	798	813	eddkdddddddddkke
13741	13756	GTGCTCGCTGAGGTCG	44	529920	67	798	813	eeeeddddddddddke
13762	13778	GCCGGCTCTGCTCATCC	427	529271	43	819	835	eekdddddddddkeke
13772	13788	TGCGCCACCCGCCGGCT	428	529272	0	829	845	eekdddddddddkeke
13776	13792	GACCTGCGCCACCCGCC	429	529273	62	833	849	eekdddddddddkeke
13777	13793	TGACCTGCGCCACCCGC	430	529274	78	834	850	eekdddddddddkeke
13836	13852	CAGGCGGAGCAGCGCGA	431	529275	70	893	909	eekdddddddddkeke
13891	13907	TCCGTTGCGGCAGGCAG	432	529276	73	948	964	eekdddddddddkeke
14017	14033	CCTGGGTCATCAGCCGG	433	529277	71	1074	1090	eekdddddddddkeke
14020	14036	AGTCCTGGGTCATCAGC	434	529278	72	1077	1093	eekdddddddddkeke
14023	14039	GGCAGTCCTGGGTCATC	435	529279	10	1080	1096	eekdddddddddkeke
14074	14090	ACATGTACTCCGTGATA	436	529280	11	1131	1147	eekdddddddddkeke
14075	14091	AACATGTACTCCGTGAT	437	529281	82	1132	1148	eekdddddddddkeke
14077	14093	AGAACATGTACTCCGTG	438	529282	87	1134	1150	eekdddddddddkeke
14077	14092	GAACATGTACTCCGTG	250	529803	71	1134	1149	kdddddddddkekee
14077	14092	GAACATGTACTCCGTG	250	529822	72	1134	1149	kdddddddddkdkee
14078	14093	AGAACATGTACTCCGT	439	529724	76	1135	1150	keddddddddddkeke
14078	14093	AGAACATGTACTCCGT	439	529744	81	1135	1150	ekdddddddddkeke
14078	14093	AGAACATGTACTCCGT	439	529764	65	1135	1150	keddddddddddkdke
14078	14093	AGAACATGTACTCCGT	439	529784	68	1135	1150	ekdddddddddkdke
14078	14094	CAGAACATGTACTCCGT	440	529853	64	1135	1151	kdeddddddddddkdke
14078	14094	CAGAACATGTACTCCGT	440	529873	69	1135	1151	edkdddddddddkdke
14078	14094	CAGAACATGTACTCCGT	440	529893	45	1135	1151	kdddkdddddksddkde
14078	14094	CAGAACATGTACTCCGT	440	529937	81	1135	1151	eekdddddddddkeke
14079	14094	CAGAACATGTACTCCG	48	529551	88	1136	1151	eekdddddddddkke
14079	14094	CAGAACATGTACTCCG	48	529576	71	1136	1151	keeddddddddddkke
14079	14094	CAGAACATGTACTCCG	48	529601	74	1136	1151	edkdddddddddkke
14079	14094	CAGAACATGTACTCCG	48	529626	72	1136	1151	kdeddddddddddkke
14079	14094	CAGAACATGTACTCCG	48	529651	85	1136	1151	kddkdddddddddkke
14079	14094	CAGAACATGTACTCCG	48	529676	67	1136	1151	kddeddddddddddkke
14079	14094	CAGAACATGTACTCCG	48	529701	82	1136	1151	eddkdddddddddkke

14079	14094	CAGAACATGTACTCCG	48	529913	76	1136	1151	eeeeddddddddkke
14090	14105	AGTAGCCGGCACAGAA	441	529811	56	1147	1162	kddddddddddkeee
14090	14105	AGTAGCCGGCACAGAA	441	529830	46	1147	1162	kddddddddddkdkke
14091	14106	GAGTAGCCGGCACAGA	442	529732	63	1148	1163	kedddddddddddkeke
14091	14106	GAGTAGCCGGCACAGA	442	529752	72	1148	1163	ekdddddddddddkkeke
14091	14106	GAGTAGCCGGCACAGA	442	529772	61	1148	1163	kedddddddddddkdkke
14091	14106	GAGTAGCCGGCACAGA	442	529792	68	1148	1163	ekddddddddddkdkke
14091	14107	CGAGTAGCCGGCACAGA	443	529861	54	1148	1164	kdedddddddddddkdkke
14091	14107	CGAGTAGCCGGCACAGA	443	529881	78	1148	1164	edkddddddddddkdkke
14091	14107	CGAGTAGCCGGCACAGA	443	529901	29	1148	1164	kdddkdddkddkdddkke
14091	14107	CGAGTAGCCGGCACAGA	443	529939	67	1148	1164	eekddddddddddkeke
14092	14108	CCGAGTAGCCGGCACAG	444	529283	70	1149	1165	eekddddddddddkeke
14092	14107	CGAGTAGCCGGCACAG	49	529552	72	1149	1164	eekddddddddddkkeke
14092	14107	CGAGTAGCCGGCACAG	49	529577	80	1149	1164	keedddddddddddkke
14092	14107	CGAGTAGCCGGCACAG	49	529602	64	1149	1164	edkddddddddddkkeke
14092	14107	CGAGTAGCCGGCACAG	49	529627	56	1149	1164	kdedddddddddddkkeke
14092	14107	CGAGTAGCCGGCACAG	49	529652	57	1149	1164	kddkddddddddddkkeke
14092	14107	CGAGTAGCCGGCACAG	49	529677	43	1149	1164	kddedddddddddddkkeke
14092	14107	CGAGTAGCCGGCACAG	49	529702	54	1149	1164	eddkddddddddddkkeke
14092	14107	CGAGTAGCCGGCACAG	49	529921	42	1149	1164	eeeeddddddddkkeke
14093	14109	TCCGAGTAGCCGGCACAA	445	529284	76	1150	1166	eekddddddddddkeke
14119	14135	CCCCCTTGCAGGAGTCC	446	529285	77	1176	1192	eekddddddddddkeke
14120	14136	TCCCCCTTGCAGGAGTC	447	529286	68	1177	1193	eekddddddddddkeke
14127	14143	TCCACTGTCCCCCTTGC	448	529287	65	1184	1200	eekddddddddddkeke
14231	14246	CCCTGGTGTACACCCC	264	529719	73	1288	1303	kedddddddddddkeke
14231	14246	CCCTGGTGTACACCCC	264	529739	83	1288	1303	ekddddddddddkeke
14231	14246	CCCTGGTGTACACCCC	264	529759	63	1288	1303	kedddddddddddkdkke
14231	14246	CCCTGGTGTACACCCC	244	529779	70	1288	1303	ekddddddddddkdkke
14231	14247	ACCCTGGTGTACACCCC	449	529848	60	1288	1304	kdedddddddddddkdkke
14231	14247	ACCCTGGTGTACACCCC	449	529868	63	1288	1304	edkddddddddddkdkke
14231	14247	ACCCTGGTGTACACCCC	449	529888	53	1288	1304	kdddkdddkddkdddkke
14232	14247	ACCCTGGTGTACACCC	265	529553	81	1289	1304	eekddddddddddkkeke
14232	14247	ACCCTGGTGTACACCC	265	529578	65	1289	1304	keedddddddddddkkeke
14232	14247	ACCCTGGTGTACACCC	265	529603	60	1289	1304	edkddddddddddkkeke
14232	14247	ACCCTGGTGTACACCC	265	529628	59	1289	1304	kdedddddddddddkkeke
14232	14247	ACCCTGGTGTACACCC	265	529653	76	1289	1304	kddkddddddddddkkeke
14232	14247	ACCCTGGTGTACACCC	265	529678	56	1289	1304	kddedddddddddddkkeke
14232	14247	ACCCTGGTGTACACCC	265	529703	68	1289	1304	eddkddddddddddkkeke
14232	14247	ACCCTGGTGTACACCC	265	529908	69	1289	1304	eeeeddddddddkkeke
14233	14249	AGACCCTGGTGTACACC	450	529168	64	1290	1306	eekddddddddddkeke
14233	14249	AGACCCTGGTGTACACC	450	529205	62	1290	1306	eekddddddddddkkeke
14240	14256	TACTGGGAGACCCTGGT	451	529290	53	1297	1313	eekddddddddddkeke
14241	14256	TACTGGGAGACCCTGG	452	529802	57	1298	1313	kddddddddddkeee

14241	14256	TACTGGGAGACCCTGG	452	529821	61	1298	1313	kddddddddddkdkkee
14242	14258	TGTACTGGGAGACCCTG	453	529292	74	1299	1315	eekddddddddddkeke
14242	14257	GTACTGGGAGACCCTG	454	529723	68	1299	1314	kedddddddddddkeke
14242	14257	GTACTGGGAGACCCTG	454	529743	84	1299	1314	ekddddddddddkeke
14242	14257	GTACTGGGAGACCCTG	454	529763	64	1299	1314	kedddddddddddkdkke
14242	14257	GTACTGGGAGACCCTG	454	529783	72	1299	1314	ekddddddddddkdkke
14242	14258	TGTACTGGGAGACCCTG	453	529852	66	1299	1315	kdedddddddddddkdkke
14242	14258	TGTACTGGGAGACCCTG	453	529872	62	1299	1315	edkddddddddddkdkke
14242	14258	TGTACTGGGAGACCCTG	453	529892	43	1299	1315	kdddkdddkddkdddkke
14243	14258	TGTACTGGGAGACCCT	252	529554	80	1300	1315	eekddddddddddkdkke
14243	14258	TGTACTGGGAGACCCT	252	529579	83	1300	1315	keedddddddddddkdkke
14243	14258	TGTACTGGGAGACCCT	252	529604	73	1300	1315	edkddddddddddkdkke
14243	14258	TGTACTGGGAGACCCT	252	529629	64	1300	1315	kdedddddddddddkdkke
14243	14258	TGTACTGGGAGACCCT	252	529654	69	1300	1315	kddkddddddddddkdkke
14243	14258	TGTACTGGGAGACCCT	252	529679	52	1300	1315	kddedddddddddddkdkke
14243	14258	TGTACTGGGAGACCCT	252	529704	63	1300	1315	eddkddddddddddkdkke
14243	14258	TGTACTGGGAGACCCT	252	529912	64	1300	1315	eeedddddddddddkdkke
14246	14262	TCGATGTACTGGGAGAC	455	529294	74	1303	1319	eekddddddddddkeke
14247	14263	CTCGATGTACTGGGAGA	456	529296	52	1304	1320	eekddddddddddkeke
14262	14278	GAGCTTTTGCAGCCACT	457	529298	60	1319	1335	eekddddddddddkeke
14263	14279	TGAGCTTTTGCAGCCAC	458	529300	71	1320	1336	eekddddddddddkeke
14349	14365	GCCTTGGCTTTCTCTCC	459	529188	79	1406	1422	eekddddddddddkeke
14349	14365	GCCTTGGCTTTCTCTCC	459	529225	78	1406	1422	eekddddddddddkdkke
14350	14365	GCCTTGGCTTTCTCTC	460	529131	58	1407	1422	eeedddddddddddkkk
14350	14365	GCCTTGGCTTTCTCTC	460	529151	71	1407	1422	kkkdddddddddddeee
14611	14627	CCCCTCTGTCCAGCGCC	461	529302	74	1668	1684	eekddddddddddkeke
14612	14628	GCCCCCTCTGTCCAGCGC	222	529189	64	1669	1685	eekddddddddddkeke
14612	14628	GCCCCCTCTGTCCAGCGC	222	529226	50	1669	1685	eekddddddddddkdkke
14613	14628	GCCCCCTCTGTCCAGCG	462	529132	78	1670	1685	eeedddddddddddkkk
14613	14628	GCCCCCTCTGTCCAGCG	462	529152	62	1670	1685	kkkdddddddddddeee
14613	14629	TGCCCCCTCTGTCCAGCG	223	529190	76	1670	1686	eekddddddddddkeke
14613	14629	TGCCCCCTCTGTCCAGCG	250	529227	88	1670	1686	eekddddddddddkdkke
14614	14629	TGCCCCCTCTGTCCAGC	463	529133	81	1671	1686	eeedddddddddddkkk
14614	14629	TGCCCCCTCTGTCCAGC	463	529153	68	1671	1686	kkkdddddddddddeee
14614	14630	CTGCCCCCTCTGTCCAGC	224	529191	78	1671	1687	eekddddddddddkeke
14614	14630	CTGCCCCCTCTGTCCAGC	224	529228	85	1671	1687	eekddddddddddkdkke
14615	14630	CTGCCCCCTCTGTCCAG	464	529134	75	1672	1687	eeedddddddddddkkk
14615	14630	CTGCCCCCTCTGTCCAG	464	529154	61	1672	1687	kkkdddddddddddeee
14707	14723	GAGGCCAGCAGATCACG	465	529304	89	1764	1780	eekddddddddddkeke
14712	14728	AGCCTGAGGCCAGCAGA	466	529306	84	1769	1785	eekddddddddddkeke
14735	14751	TCCAGCAATGAAGGCAG	467	529308	68	1792	1808	eekddddddddddkeke
14739	14755	TGTCTCCAGCAATGAAG	468	529310	59	1796	1812	eekddddddddddkeke
15099	15115	CACATGGAGTCAGCATC	469	529169	79	2156	2172	eekddddddddddkeke

15099	15115	CACATGGAGTCAGCATC	469	529206	82	2156	2172	eekdddddddddkkke
15100	15116	ACACATGGAGTCAGCAT	470	529312	68	2157	2173	eekdddddddddkkeke
15109	15125	GAGGACAGCACACATGG	471	529314	61	2166	2182	eekdddddddddkkeke
15128	15144	AGCTAAACAACCGCCTT	472	529316	62	2185	2201	eekdddddddddkkeke
15128	15143	GCTAAACAACCGCCTT	59	529555	78	2185	2200	eekdddddddddkke
15128	15143	GCTAAACAACCGCCTT	59	529580	73	2185	2200	keeddddddddddkke
15128	15143	GCTAAACAACCGCCTT	59	529605	71	2185	2200	edkdddddddddkke
15128	15143	GCTAAACAACCGCCTT	59	529630	64	2185	2200	kdeddddddddddkke
15128	15143	GCTAAACAACCGCCTT	59	529655	63	2185	2200	kdkkdddddddddkke
15128	15143	GCTAAACAACCGCCTT	59	529680	43	2185	2200	kddeddddddddddkke
15128	15143	GCTAAACAACCGCCTT	59	529705	63	2185	2200	eddkdddddddddkke
15128	15143	GCTAAACAACCGCCTT	59	529932	60	2185	2200	eeeedddddddddkke
15129	15145	GAGCTAAACAACCGCCT	473	529318	82	2186	2202	eekdddddddddkkeke
15130	15146	AGAGCTAAACAACCGCC	474	529170	85	2187	2203	eekdddddddddkkeke
15130	15146	AGAGCTAAACAACCGCC	474	529207	88	2187	2203	eekdddddddddkkke
15131	15147	GAGAGCTAAACAACCGC	475	529171	81	2188	2204	eekdddddddddkkeke
15131	15147	GAGAGCTAAACAACCGC	475	529208	84	2188	2204	eekdddddddddkkke
15162	15177	AAGATGATAATGGATA	476	529805	40	2219	2234	kdddddddddkkeke
15162	15177	AAGATGATAATGGATA	476	529824	32	2219	2234	kdddddddddkdkee
15163	15179	TGAAGATGATAATGGAT	477	529320	74	2220	2236	eekdddddddddkkeke
15163	15178	GAAGATGATAATGGAT	478	529726	80	2220	2235	keddddddddddkkeke
15163	15178	GAAGATGATAATGGAT	478	529746	82	2220	2235	ekdddddddddkkeke
15163	15178	GAAGATGATAATGGAT	478	529766	63	2220	2235	kedddddddddkdke
15163	15178	GAAGATGATAATGGAT	478	529786	69	2220	2235	ekdddddddddkdke
15163	15179	TGAAGATGATAATGGAT	477	529855	39	2220	2236	kdedddddddddkdke
15163	15179	TGAAGATGATAATGGAT	477	529875	40	2220	2236	edkddddddddkdke
15163	15179	TGAAGATGATAATGGAT	477	529895	27	2220	2236	kdddkdddkdddkke
15164	15179	TGAAGATGATAATGGA	61	529556	72	2221	2236	eekdddddddddkke
15164	15179	TGAAGATGATAATGGA	61	529581	68	2221	2236	keeddddddddddkke
15164	15179	TGAAGATGATAATGGA	61	529606	54	2221	2236	edkdddddddddkke
15164	15179	TGAAGATGATAATGGA	61	529631	29	2221	2236	kdeddddddddddkke
15164	15179	TGAAGATGATAATGGA	61	529656	74	2221	2236	kdkkdddddddddkke
15164	15179	TGAAGATGATAATGGA	61	529681	32	2221	2236	kddeddddddddddkke
15164	15179	TGAAGATGATAATGGA	61	529706	41	2221	2236	eddkdddddddddkke
15164	15179	TGAAGATGATAATGGA	61	529915	51	2221	2236	eeeedddddddddkke
15182	15198	GCTTCTGAATTGTCTGA	226	529172	88	2239	2255	eekdddddddddkkeke
15182	15198	GCTTCTGAATTGTCTGA	226	529209	87	2239	2255	eekdddddddddkkke
15183	15199	TGCTTCTGAATTGTCTG	227	529173	92	2240	2256	eekdddddddddkkeke
15183	15199	TGCTTCTGAATTGTCTG	227	529210	89	2240	2256	eekdddddddddkkke
15184	15200	ATGCTTCTGAATTGTCT	479	529183	85	2241	2257	eekdddddddddkkeke
15184	15200	ATGCTTCTGAATTGTCT	479	529220	92	2241	2257	eekdddddddddkkke
15185	15200	ATGCTTCTGAATTGTC	257	529126	83	2242	2257	eeedddddddddkkk
15185	15200	ATGCTTCTGAATTGTC	257	529146	84	2242	2257	kkkdddddddddeee

15185	15201	GATGCTTCTGAATTGTC	480	529174	85	2242	2258	eekddddddddddkkeke
15185	15201	GATGCTTCTGAATTGTC	480	529211	86	2242	2258	eekddddddddddkkke
15188	15204	GGTGATGCTTCTGAATT	481	529322	71	2245	2261	eekddddddddddkkeke
15189	15205	TGGTGATGCTTCTGAAT	482	529324	79	2246	2262	eekddddddddddkkeke
15190	15206	ATGGTGATGCTTCTGAA	483	529326	85	2247	2263	eekddddddddddkkeke
15191	15207	CATGGTGATGCTTCTGA	228	529175	92	2248	2264	eekddddddddddkkeke
15191	15207	CATGGTGATGCTTCTGA	228	529212	92	2248	2264	eekddddddddddkkke
15192	15208	GCATGGTGATGCTTCTG	229	529176	89	2249	2265	eekddddddddddkkeke
15192	15208	GCATGGTGATGCTTCTG	229	529213	90	2249	2265	eekddddddddddkkke
15192	15207	CATGGTGATGCTTCTG	259	529804	89	2249	2264	kddddddddddkkekee
15192	15207	CATGGTGATGCTTCTG	259	529823	89	2249	2264	kddddddddddkdkkee
15193	15209	TGCATGGTGATGCTTCT	230	529166	83	2250	2266	eekddddddddddkkeke
15193	15209	TGCATGGTGATGCTTCT	230	529203	86	2250	2266	eekddddddddddkkke
15193	15208	GCATGGTGATGCTTCT	260	529725	92	2250	2265	kedddddddddddkkeke
15193	15208	GCATGGTGATGCTTCT	260	529745	91	2250	2265	ekddddddddddkkeke
15193	15208	GCATGGTGATGCTTCT	260	529765	88	2250	2265	kedddddddddddkdke
15193	15208	GCATGGTGATGCTTCT	260	529785	91	2250	2265	ekddddddddddkdkke
15193	15208	GCATGGTGATGCTTCT	260	529799	89	2250	2265	kddddddddddkkekee
15193	15208	GCATGGTGATGCTTCT	260	529818	88	2250	2265	kddddddddddkdkkee
15193	15209	TGCATGGTGATGCTTCT	230	529854	90	2250	2266	kdedddddddddddkdkke
15193	15209	TGCATGGTGATGCTTCT	230	529874	81	2250	2266	edkddddddddddkdkke
15193	15209	TGCATGGTGATGCTTCT	230	529894	60	2250	2266	kdddkdddkddkdddkke
15194	15210	ATGCATGGTGATGCTTC	231	529167	71	2251	2267	eekddddddddddkkeke
15194	15210	ATGCATGGTGATGCTTC	231	529204	70	2251	2267	eekddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529557	86	2251	2266	eekddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529582	86	2251	2266	keedddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529607	84	2251	2266	edkddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529632	81	2251	2266	kdedddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529657	85	2251	2266	kddkddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529682	78	2251	2266	kddedddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529707	79	2251	2266	eddkddddddddddkkke
15194	15209	TGCATGGTGATGCTTC	69	529720	75	2251	2266	kedddddddddddkkeke
15194	15209	TGCATGGTGATGCTTC	69	529740	70	2251	2266	ekddddddddddkkeke
15194	15209	TGCATGGTGATGCTTC	69	529760	78	2251	2266	kedddddddddddkdkke
15194	15209	TGCATGGTGATGCTTC	69	529780	83	2251	2266	ekddddddddddkdkke
15194	15210	ATGCATGGTGATGCTTC	231	529849	80	2251	2267	kdedddddddddddkdkke
15194	15210	ATGCATGGTGATGCTTC	231	529869	72	2251	2267	edkddddddddddkdkke
15194	15210	ATGCATGGTGATGCTTC	231	529889	49	2251	2267	kdddkdddkddkdddkke
15194	15209	TGCATGGTGATGCTTC	69	529914	69	2251	2266	eeeedddddddddkkke
15195	15211	CATGCATGGTGATGCTT	484	529328	68	2252	2268	eekddddddddddkkeke
15195	15210	ATGCATGGTGATGCTT	71	529558	71	2252	2267	eekddddddddddkkke
15195	15210	ATGCATGGTGATGCTT	71	529583	81	2252	2267	keedddddddddddkkke
15195	15210	ATGCATGGTGATGCTT	71	529608	68	2252	2267	edkddddddddddkkke

15195	15210	ATGCATGGTGATGCTT	71	529633	73	2252	2267	kdeddddddddkke
15195	15210	ATGCATGGTGATGCTT	71	529658	63	2252	2267	kddkdddddddkke
15195	15210	ATGCATGGTGATGCTT	71	529683	74	2252	2267	kdeddddddddkke
15195	15210	ATGCATGGTGATGCTT	71	529708	70	2252	2267	eddkdddddddkke
15195	15210	ATGCATGGTGATGCTT	71	529909	59	2252	2267	eeeddddddddkke
15205	15221	CATTCGCCACCATGCAT	485	529192	51	2262	2278	eekdddddddkke
15205	15221	CATTCGCCACCATGCAT	485	529229	69	2262	2278	eekdddddddkkke
15206	15221	CATTCGCCACCATGCA	486	529135	54	2263	2278	eeeddddddddkk
15206	15221	CATTCGCCACCATGCA	486	529155	56	2263	2278	kkkdddddddeee
15805	15821	TGGTGCCAGGACGGCC	487	529330	37	2862	2878	eekdddddddkke

Example 20: Design of modified antisense oligonucleotides comprising MOE and cEt modifications targeting human coagulation Factor VII

Based on the activity of the antisense oligonucleotides listed above, additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid at start positions 1147, 1154, or 12842 of SEQ ID NO: 1. The newly designed modified antisense oligonucleotides and their motifs are described in Table 21. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The ‘Sugar Chemistry’ column provides the sugar modifications throughout each oligonucleotide: ‘d’ indicates a 2’-deoxynucleoside, ‘k’ indicates a constrained ethyl (cEt) nucleoside, and ‘e’ indicates a 2’-O-methoxyethyl nucleoside. The ‘Sequence’ column provides the nucleobase sequence for each SEQ ID NO.

Each oligonucleotide listed in Table 21 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. “Start site” indicates the 5’-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. “Stop site” indicates the 3’-most nucleoside to which the oligonucleotide is targeted human gene sequence. ‘n/a’ indicates that the antisense oligonucleotide is not 100% complementary with that particular gene sequence.

Table 21
Chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Sugar Chemistry
1147	1162	GATGAAATCTCTGCAG	21	529544	36	51	eekdddddddkke
1147	1162	GATGAAATCTCTGCAG	21	529569	36	51	keeddddddddkke
1147	1162	GATGAAATCTCTGCAG	21	529594	36	51	edkdddddddkke
1147	1162	GATGAAATCTCTGCAG	21	529619	36	51	kdeddddddddkke
1147	1162	GATGAAATCTCTGCAG	21	529644	36	51	kddkdddddddkke
1147	1162	GATGAAATCTCTGCAG	21	529669	36	51	kdeddddddddkke
1147	1162	GATGAAATCTCTGCAG	21	529694	36	51	eddkdddddddkke

1147	1162	GATGAAATCTCTGCAG	21	529929	36	51	eeeeeeeeeeeekkke
1152	1167	ACCATGATGAAATCTC	488	529809	41	56	kdeeeeeeeeeekkee
1152	1167	ACCATGATGAAATCTC	488	529828	41	56	kdeeeeeeeeeekkee
1153	1168	GACCATGATGAAATCT	489	529730	42	57	kedeeeeeeeeekkee
1153	1168	GACCATGATGAAATCT	489	529750	42	57	ekdeeeeeeeeeekkee
1153	1168	GACCATGATGAAATCT	489	529770	42	57	kedeeeeeeeeekkee
1153	1168	GACCATGATGAAATCT	489	529790	42	57	ekdeeeeeeeeeekkee
1153	1169	AGACCATGATGAAATCT	490	529859	42	58	kdeeeeeeeeeekkee
1153	1169	AGACCATGATGAAATCT	490	529879	42	58	edkdeeeeeeeeeekkee
1153	1169	AGACCATGATGAAATCT	490	529899	42	58	kdeeeekdeeeekdeee
1154	1169	AGACCATGATGAAATC	22	529545	43	58	eekeeeeeeeeekkke
1154	1169	AGACCATGATGAAATC	22	529570	43	58	keeeeeeeeeeeeekkke
1154	1169	AGACCATGATGAAATC	22	529595	43	58	edkdeeeeeeeeekkke
1154	1169	AGACCATGATGAAATC	22	529620	43	58	kdeeeeeeeeeekkee
1154	1169	AGACCATGATGAAATC	22	529645	43	58	kdeeeekdeeeekdeee
1154	1169	AGACCATGATGAAATC	22	529670	43	58	kdeeeeeeeeeekkee
1154	1169	AGACCATGATGAAATC	22	529695	43	58	edkdeeeeeeeeekkke
1154	1169	AGACCATGATGAAATC	22	529919	43	58	eeeeeeeeeeeekkke
12842	12857	CCACCCAGATGGTGTT	41	529548	715	730	eekeeeeeeeeekkke
12842	12857	CCACCCAGATGGTGTT	41	529573	715	730	keeeeeeeeeeeeekkke
12842	12857	CCACCCAGATGGTGTT	41	529598	715	730	edkdeeeeeeeeekkke
12842	12857	CCACCCAGATGGTGTT	41	529623	715	730	kdeeeeeeeeeekkee
12842	12857	CCACCCAGATGGTGTT	41	529648	715	730	kdeeeekdeeeekdeee
12842	12857	CCACCCAGATGGTGTT	41	529673	715	730	kdeeeeeeeeeekkee
12842	12857	CCACCCAGATGGTGTT	41	529698	715	730	edkdeeeeeeeeekkke
12842	12857	CCACCCAGATGGTGTT	41	529930	715	730	eeeeeeeeeeeekkke

Example 21: Modified antisense oligonucleotides comprising cEt and MOE modifications targeting human coagulation Factor VII

Additional antisense oligonucleotides were designed targeting a Factor VII nucleic acid and were tested for their effects on Factor VII mRNA in vitro. ISIS 472998, a 2-10-2 cEt gapmer, and ISIS 515554, a deoxy, MOE, and cEt oligonucleotide, described in the Examples above were also included in the screen.

The newly designed modified antisense oligonucleotides and their motifs are described in Table 22. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The 'Sugar Chemistry' column provides the sugar modifications throughout each oligonucleotide: 'd' indicates a 2'-deoxynucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'e' indicates a 2'-O-methoxyethyl nucleoside. The 'Sequence' column provides the nucleobase sequence for each SEQ ID NO.

Each oligonucleotide listed in Table 22 is targeted to either the human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides

1255000 to 1273000) or the human Factor VII mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_019616.2), or both. "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. 'n/a' indicates that the antisense

5 oligonucleotide is not 100% complementary with that particular gene sequence.

Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 (described hereinabove in Example 1) was used to measure mRNA levels. Factor VII

10 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

Table 22

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1 and SEQ ID NO: 2

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	% inhibition	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Sugar Chemistry
15263	15276	TGTGCAGCCCGGCA	74	472998	88	2320	2333	kkdddddiddk
2499	2514	GCAGATTTGCATCAGA	493	515554	75	n/a	n/a	eeddddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534530	92	n/a	n/a	kekeddddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534563	92	n/a	n/a	kekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534596	88	n/a	n/a	ekedddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534629	89	n/a	n/a	ekeddddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534662	87	n/a	n/a	eekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534695	92	n/a	n/a	eekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534732	90	n/a	n/a	ekekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534767	92	n/a	n/a	keekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534802	93	n/a	n/a	ekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534832	83	n/a	n/a	edkdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534862	72	n/a	n/a	kdeddddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534892	82	n/a	n/a	eekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534922	80	n/a	n/a	kddkdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534952	72	n/a	n/a	kdeddddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	534982	77	n/a	n/a	eddkdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	535012	70	n/a	n/a	eeeddddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	535045	84	n/a	n/a	eeeddddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	535078	87	n/a	n/a	eeekdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	535111	63	n/a	n/a	eeeedddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	535144	69	n/a	n/a	ededkdddiddk
2238	2253	ACCAGTGGCAGTCCCT	491	535177	68	n/a	n/a	edkdeddddiddk
2492	2507	TGCATCAGAAAAGCTC	492	534531	61	n/a	n/a	kekdddiddk

2492	2507	TGCATCAGAAAAGCTC	492	534564	30	n/a	n/a	kekdddddddekek
2492	2507	TGCATCAGAAAAGCTC	492	534597	67	n/a	n/a	ekeeddddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	534630	54	n/a	n/a	ekeddddddddekke
2492	2507	TGCATCAGAAAAGCTC	492	534663	94	n/a	n/a	eekkkdddddddeke
2492	2507	TGCATCAGAAAAGCTC	492	534696	68	n/a	n/a	eekddddddddkcke
2492	2507	TGCATCAGAAAAGCTC	492	534733	44	n/a	n/a	ekekdddddddkcke
2492	2507	TGCATCAGAAAAGCTC	492	534768	55	n/a	n/a	keekdddddddkcek
2492	2507	TGCATCAGAAAAGCTC	492	534803	73	n/a	n/a	ekkkdddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	534833	65	n/a	n/a	edkdddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	534863	53	n/a	n/a	kdeddddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	534893	61	n/a	n/a	eekdddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	534923	70	n/a	n/a	kdkkdddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	534953	54	n/a	n/a	kdeddddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	534983	58	n/a	n/a	eddkdddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	535013	52	n/a	n/a	eeeeddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	535046	67	n/a	n/a	eeeeddddddkk
2492	2507	TGCATCAGAAAAGCTC	492	535079	57	n/a	n/a	eeekddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	535112	42	n/a	n/a	eeeeddddddkke
2492	2507	TGCATCAGAAAAGCTC	492	535145	41	n/a	n/a	ededkdddddkke
2492	2507	TGCATCAGAAAAGCTC	492	535178	35	n/a	n/a	edkdeddddkke
2498	2513	CAGATTTGCATCAGAA	493	534565	87	n/a	n/a	kekdddddddekek
2498	2513	CAGATTTGCATCAGAA	493	534598	72	n/a	n/a	ekeeddddddkke
2498	2513	CAGATTTGCATCAGAA	493	534631	70	n/a	n/a	ekeddddddekke
2498	2513	CAGATTTGCATCAGAA	493	534664	94	n/a	n/a	eekkkdddddeke
2498	2513	CAGATTTGCATCAGAA	493	534697	90	n/a	n/a	eekddddddkcke
2498	2513	CAGATTTGCATCAGAA	493	534734	74	n/a	n/a	ekekdddddkcke
2498	2513	CAGATTTGCATCAGAA	493	534769	80	n/a	n/a	keekdddddkcek
2498	2513	CAGATTTGCATCAGAA	493	534804	87	n/a	n/a	ekkkdddddkke
2498	2513	CAGATTTGCATCAGAA	493	534834	76	n/a	n/a	edkdddddddkke
2498	2513	CAGATTTGCATCAGAA	493	534864	56	n/a	n/a	kdeddddddkke
2498	2513	CAGATTTGCATCAGAA	493	534894	67	n/a	n/a	eekdddddddkke
2498	2513	CAGATTTGCATCAGAA	493	534924	71	n/a	n/a	kdkkdddddkke
2498	2513	CAGATTTGCATCAGAA	493	534954	54	n/a	n/a	kdeddddddkke
2498	2513	CAGATTTGCATCAGAA	493	534984	48	n/a	n/a	eddkdddddkke
2498	2513	CAGATTTGCATCAGAA	493	535014	43	n/a	n/a	eeeeddddddkke
2498	2513	CAGATTTGCATCAGAA	493	535047	60	n/a	n/a	eeeeddddddkk
2498	2513	CAGATTTGCATCAGAA	493	535080	64	n/a	n/a	eeekdddddkke
2498	2513	CAGATTTGCATCAGAA	493	535113	32	n/a	n/a	eeeeddddddkke
2498	2513	CAGATTTGCATCAGAA	493	535146	31	n/a	n/a	ededkdddddkke
2498	2513	CAGATTTGCATCAGAA	493	535179	28	n/a	n/a	edkdeddddkke
4729	4744	GTCTGGTTTGAAGGA	494	534533	82	n/a	n/a	kekeddddddekek
4729	4744	GTCTGGTTTGAAGGA	494	534566	88	n/a	n/a	kekdddddddekek
4729	4744	GTCTGGTTTGAAGGA	494	534599	65	n/a	n/a	ekeeddddddkke

4729	4744	GTCTGGTTTGGGAAGGA	494	534632	69	n/a	n/a	ekeddddddddekke
4729	4744	GTCTGGTTTGGGAAGGA	494	534665	87	n/a	n/a	eekdddddddeke
4729	4744	GTCTGGTTTGGGAAGGA	494	534698	64	n/a	n/a	eekdddddddeke
4729	4744	GTCTGGTTTGGGAAGGA	494	534735	63	n/a	n/a	eekdddddddeke
4729	4744	GTCTGGTTTGGGAAGGA	494	534770	66	n/a	n/a	keekdddddddeke
4729	4744	GTCTGGTTTGGGAAGGA	494	534805	87	n/a	n/a	ekkkdddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	534835	68	n/a	n/a	edkdddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	534865	66	n/a	n/a	kdeddddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	534895	57	n/a	n/a	eekdddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	534925	82	n/a	n/a	kdkdddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	534955	76	n/a	n/a	kddeddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	534985	71	n/a	n/a	eddkdddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	535015	59	n/a	n/a	eeeeddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	535048	69	n/a	n/a	eeeeddddddkk
4729	4744	GTCTGGTTTGGGAAGGA	494	535081	67	n/a	n/a	eeekdddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	535114	37	n/a	n/a	eeeeddddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	535147	32	n/a	n/a	ededkdddddkke
4729	4744	GTCTGGTTTGGGAAGGA	494	535180	31	n/a	n/a	edkdeddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534534	94	n/a	n/a	kekeddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534567	92	n/a	n/a	kekdddddddekek
4851	4866	GGTACTGAGCGCGGA	234	534600	92	n/a	n/a	ekeeddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534633	91	n/a	n/a	ekeddddddddekke
4851	4866	GGTACTGAGCGCGGA	234	534666	89	n/a	n/a	eekkkdddddeke
4851	4866	GGTACTGAGCGCGGA	234	534699	91	n/a	n/a	eekdddddddeke
4851	4866	GGTACTGAGCGCGGA	234	534736	83	n/a	n/a	eekkkdddddeke
4851	4866	GGTACTGAGCGCGGA	234	534771	80	n/a	n/a	keekdddddddeke
4851	4866	GGTACTGAGCGCGGA	234	534806	96	n/a	n/a	ekkkdddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534836	86	n/a	n/a	edkdddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534866	82	n/a	n/a	kdeddddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534896	82	n/a	n/a	eekdddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534926	89	n/a	n/a	kdkdddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534956	91	n/a	n/a	kddeddddddkke
4851	4866	GGTACTGAGCGCGGA	234	534986	87	n/a	n/a	eddkdddddddkke
4851	4866	GGTACTGAGCGCGGA	234	535016	83	n/a	n/a	eeeeddddddkke
4851	4866	GGTACTGAGCGCGGA	234	535049	87	n/a	n/a	eeeeddddddkk
4851	4866	GGTACTGAGCGCGGA	234	535082	87	n/a	n/a	eeekdddddddkke
4851	4866	GGTACTGAGCGCGGA	234	535115	77	n/a	n/a	eeeeddddddkke
4851	4866	GGTACTGAGCGCGGA	234	535148	73	n/a	n/a	ededkdddddkke
4851	4866	GGTACTGAGCGCGGA	234	535181	68	n/a	n/a	edkdeddddddkke
4873	4888	TTCTGCAGGAGCGGCC	236	534535	66	n/a	n/a	kekeddddddkke
4873	4888	TTCTGCAGGAGCGGCC	236	534568	85	n/a	n/a	kekdddddddekek
4873	4888	TTCTGCAGGAGCGGCC	236	534601	51	n/a	n/a	ekeeddddddkke
4873	4888	TTCTGCAGGAGCGGCC	236	534634	80	n/a	n/a	ekeddddddddekke

4873	4888	TTCTGCAGGAGCGGCC	236	534667	90	n/a	n/a	eekkkkkkkkkkkkeke
4873	4888	TTCTGCAGGAGCGGCC	236	534700	88	n/a	n/a	eekkkkkkkkkkkkeke
4873	4888	TTCTGCAGGAGCGGCC	236	534737	65	n/a	n/a	ekkkkkkkkkkkkeke
4873	4888	TTCTGCAGGAGCGGCC	236	534772	77	n/a	n/a	keekkkkkkkkkkkeek
4873	4888	TTCTGCAGGAGCGGCC	236	534807	84	n/a	n/a	ekkkkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	534837	78	n/a	n/a	edkkkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	534867	44	n/a	n/a	kdedkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	534897	82	n/a	n/a	eekkkkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	534927	61	n/a	n/a	kddkkkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	534957	58	n/a	n/a	kdedkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	534987	49	n/a	n/a	eddkkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	535017	38	n/a	n/a	eeekkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	535050	32	n/a	n/a	eeekkkkkkkkkkkkk
4873	4888	TTCTGCAGGAGCGGCC	236	535083	43	n/a	n/a	eekkkkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	535116	9	n/a	n/a	eeekkkkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	535149	23	n/a	n/a	ededkkkkkkkkkkke
4873	4888	TTCTGCAGGAGCGGCC	236	535182	18	n/a	n/a	edkdedkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534536	89	n/a	n/a	kekdkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534569	90	n/a	n/a	kekdkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534602	85	n/a	n/a	ekedkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534635	87	n/a	n/a	ekedkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534668	90	n/a	n/a	eekkkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534701	92	n/a	n/a	eekkkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534738	81	n/a	n/a	ekkkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534773	79	n/a	n/a	keekkkkkkkkkkkeek
5512	5527	CCGAGGCGCGGCCCT	238	534808	90	n/a	n/a	ekkkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534838	88	n/a	n/a	edkkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534868	67	n/a	n/a	kdedkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534898	89	n/a	n/a	eekkkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534928	81	n/a	n/a	kddkkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534958	78	n/a	n/a	kdedkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	534988	66	n/a	n/a	eddkkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	535018	78	n/a	n/a	eeekkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	535051	76	n/a	n/a	eeekkkkkkkkkkkkk
5512	5527	CCGAGGCGCGGCCCT	238	535084	80	n/a	n/a	eeekkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	535117	58	n/a	n/a	eeekkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	535150	51	n/a	n/a	ededkkkkkkkkkkke
5512	5527	CCGAGGCGCGGCCCT	238	535183	53	n/a	n/a	edkdedkkkkkkkkke
5513	5528	TCCGAGGCGCGGCCCC	239	534537	91	n/a	n/a	kekdkkkkkkkkkkkke
5513	5528	TCCGAGGCGCGGCCCC	239	534570	85	n/a	n/a	kekdkkkkkkkkkkkke
5513	5528	TCCGAGGCGCGGCCCC	239	534603	79	n/a	n/a	ekedkkkkkkkkkkke
5513	5528	TCCGAGGCGCGGCCCC	239	534636	72	n/a	n/a	ekedkkkkkkkkkkke
5513	5528	TCCGAGGCGCGGCCCC	239	534669	85	n/a	n/a	eekkkkkkkkkkkkkke

5513	5528	TCCGAGGCGCGGCCCC	239	534702	85	n/a	n/a	eekdddddddddkeke
5513	5528	TCCGAGGCGCGGCCCC	239	534739	73	n/a	n/a	ekeddddddddddkeke
5513	5528	TCCGAGGCGCGGCCCC	239	534774	77	n/a	n/a	keekdddddddddkeek
5513	5528	TCCGAGGCGCGGCCCC	239	534809	91	n/a	n/a	ekkdddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	534839	86	n/a	n/a	edkdddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	534869	71	n/a	n/a	kdeddddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	534899	82	n/a	n/a	eekdddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	534929	83	n/a	n/a	kdkdddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	534959	80	n/a	n/a	kdeddddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	534989	79	n/a	n/a	edkdddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	535019	76	n/a	n/a	eeeeddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	535052	79	n/a	n/a	eeeeddddddddkkk
5513	5528	TCCGAGGCGCGGCCCC	239	535085	81	n/a	n/a	eeekdddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	535118	58	n/a	n/a	eeeeddddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	535151	65	n/a	n/a	ededkdddddddkke
5513	5528	TCCGAGGCGCGGCCCC	239	535184	60	n/a	n/a	edkdeddddddddkke
6077	6092	CCCGGCCGCAGTCCT	495	534516	77	182	197	kekeddddddddkek
6077	6092	CCCGGCCGCAGTCCT	495	534549	80	182	197	kekddddddddekek
6077	6092	CCCGGCCGCAGTCCT	495	534582	73	182	197	ekeddddddddkke
6077	6092	CCCGGCCGCAGTCCT	495	534615	79	182	197	ekeddddddddkekke
6077	6092	CCCGGCCGCAGTCCT	495	534648	67	182	197	eekddddddddeke
6077	6092	CCCGGCCGCAGTCCT	495	534681	87	182	197	eekdddddddkeke
6077	6092	CCCGGCCGCAGTCCT	495	534718	46	182	197	ekeddddddddkeke
6077	6092	CCCGGCCGCAGTCCT	495	534753	68	182	197	keekdddddddkeek
6077	6092	CCCGGCCGCAGTCCT	495	534788	84	182	197	ekkddddddddekke
6077	6092	CCCGGCCGCAGTCCT	495	534818	82	182	197	edkddddddddekke
6077	6092	CCCGGCCGCAGTCCT	495	534848	75	182	197	kdedddddddddekke
6077	6092	CCCGGCCGCAGTCCT	495	534878	72	182	197	eekddddddddekke
6077	6092	CCCGGCCGCAGTCCT	495	534908	81	182	197	kdkddddddddekke
6077	6092	CCCGGCCGCAGTCCT	495	534938	69	182	197	kdedddddddddekke
6077	6092	CCCGGCCGCAGTCCT	495	534968	77	182	197	edkddddddddekke
6077	6092	CCCGGCCGCAGTCCT	495	534998	76	182	197	eeeeddddddddkke
6077	6092	CCCGGCCGCAGTCCT	495	535031	76	182	197	eeeeddddddddkkk
6077	6092	CCCGGCCGCAGTCCT	495	535064	70	182	197	eeekdddddddkke
6077	6092	CCCGGCCGCAGTCCT	495	535097	57	182	197	eeeeddddddddkke
6077	6092	CCCGGCCGCAGTCCT	495	535130	69	182	197	ededkdddddddkke
6077	6092	CCCGGCCGCAGTCCT	495	535163	58	182	197	edkdeddddddddkke
8644	8659	AAGAAACTGTTGGCCA	241	534538	71	n/a	n/a	kekeddddddddkek
8644	8659	AAGAAACTGTTGGCCA	241	534571	64	n/a	n/a	kekddddddddekek
8644	8659	AAGAAACTGTTGGCCA	241	534604	66	n/a	n/a	ekedddddddddekke
8644	8659	AAGAAACTGTTGGCCA	241	534637	74	n/a	n/a	ekedddddddddekke
8644	8659	AAGAAACTGTTGGCCA	241	534670	87	n/a	n/a	eekddddddddeke
8644	8659	AAGAAACTGTTGGCCA	241	534703	72	n/a	n/a	eekdddddddkeke

8644	8659	AAGAAACTGTTGGCCA	241	534740	56	n/a	n/a	ekekdddddddkkeke
8644	8659	AAGAAACTGTTGGCCA	241	534775	53	n/a	n/a	keekdddddddkkeek
8644	8659	AAGAAACTGTTGGCCA	241	534810	78	n/a	n/a	ekkkdddddddkke
8644	8659	AAGAAACTGTTGGCCA	241	534840	73	n/a	n/a	edkdddddddkke
8644	8659	AAGAAACTGTTGGCCA	241	534870	65	n/a	n/a	kdeddnddddkke
8644	8659	AAGAAACTGTTGGCCA	241	534900	69	n/a	n/a	eekdddddddkke
8644	8659	AAGAAACTGTTGGCCA	241	534930	67	n/a	n/a	kdkkdddddddkke
8644	8659	AAGAAACTGTTGGCCA	241	534960	62	n/a	n/a	kddeddnddddkke
8644	8659	AAGAAACTGTTGGCCA	241	534990	66	n/a	n/a	eddkdddddddkke
8644	8659	AAGAAACTGTTGGCCA	241	535020	61	n/a	n/a	eeeednddddkke
8644	8659	AAGAAACTGTTGGCCA	241	535053	47	n/a	n/a	eeeednddddkkk
8644	8659	AAGAAACTGTTGGCCA	241	535086	61	n/a	n/a	eeeknddddkke
8644	8659	AAGAAACTGTTGGCCA	241	535119	49	n/a	n/a	eeeednddddkke
8644	8659	AAGAAACTGTTGGCCA	241	535152	48	n/a	n/a	ededknddddkke
8644	8659	AAGAAACTGTTGGCCA	241	535185	57	n/a	n/a	edkdednddddkke
8861	8876	ATGGGTGACCACACAT	496	534539	70	n/a	n/a	kekendndddkek
8861	8876	ATGGGTGACCACACAT	496	534572	82	n/a	n/a	kekndndddekek
8861	8876	ATGGGTGACCACACAT	496	534605	59	n/a	n/a	ekeendndddkke
8861	8876	ATGGGTGACCACACAT	496	534638	69	n/a	n/a	ekedndnddekke
8861	8876	ATGGGTGACCACACAT	496	534671	89	n/a	n/a	eekndndddeke
8861	8876	ATGGGTGACCACACAT	496	534704	83	n/a	n/a	eekndndddkeke
8861	8876	ATGGGTGACCACACAT	496	534741	47	n/a	n/a	ekekndndddkeke
8861	8876	ATGGGTGACCACACAT	496	534776	46	n/a	n/a	keekndnddkkeek
8861	8876	ATGGGTGACCACACAT	496	534811	71	n/a	n/a	ekkkndnddkke
8861	8876	ATGGGTGACCACACAT	496	534841	61	n/a	n/a	edkndnddddkke
8861	8876	ATGGGTGACCACACAT	496	534871	53	n/a	n/a	kdeddnddddkke
8861	8876	ATGGGTGACCACACAT	496	534901	55	n/a	n/a	eekndnddddkke
8861	8876	ATGGGTGACCACACAT	496	534931	73	n/a	n/a	kdkkndnddddkke
8861	8876	ATGGGTGACCACACAT	496	534961	53	n/a	n/a	kddeddnddddkke
8861	8876	ATGGGTGACCACACAT	496	534991	56	n/a	n/a	eddkndnddddkke
8861	8876	ATGGGTGACCACACAT	496	535021	58	n/a	n/a	eeeednddddkke
8861	8876	ATGGGTGACCACACAT	496	535054	59	n/a	n/a	eeeednddddkkk
8861	8876	ATGGGTGACCACACAT	496	535087	0	n/a	n/a	eeekndndddkke
8861	8876	ATGGGTGACCACACAT	496	535120	41	n/a	n/a	eeeednddddkke
8861	8876	ATGGGTGACCACACAT	496	535153	44	n/a	n/a	ededkndnddkke
8861	8876	ATGGGTGACCACACAT	496	535186	35	n/a	n/a	edkdedndddkke
9598	9613	AAGTTTACCAAGCGGT	497	534573	76	n/a	n/a	kekndndddekek
9598	9613	AAGTTTACCAAGCGGT	497	534606	55	n/a	n/a	ekeendndddkke
9598	9613	AAGTTTACCAAGCGGT	497	534639	72	n/a	n/a	ekedndnddekke
9598	9613	AAGTTTACCAAGCGGT	497	534672	89	n/a	n/a	eekndndddeke
9598	9613	AAGTTTACCAAGCGGT	497	534705	87	n/a	n/a	eekndndddkeke
9598	9613	AAGTTTACCAAGCGGT	497	534742	84	n/a	n/a	ekekndndddkeke
9598	9613	AAGTTTACCAAGCGGT	497	534777	79	n/a	n/a	keekndnddkkeek

9598	9613	AAGTTTACCAAGCGGT	497	534812	76	n/a	n/a	ekkkkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	534842	74	n/a	n/a	edkkkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	534872	53	n/a	n/a	kdedkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	534902	70	n/a	n/a	eekkkkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	534932	73	n/a	n/a	kdkkkkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	534962	60	n/a	n/a	kddedkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	534992	61	n/a	n/a	eddkkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	535022	38	n/a	n/a	eeeedkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	535055	42	n/a	n/a	eeeedkkkkkkkkkkk
9598	9613	AAGTTTACCAAGCGGT	497	535088	56	n/a	n/a	eeekkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	535121	5	n/a	n/a	eeeedkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	535154	22	n/a	n/a	ededkkkkkkkkkkke
9598	9613	AAGTTTACCAAGCGGT	497	535187	16	n/a	n/a	edkdedkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534541	86	n/a	n/a	kekedkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534574	89	n/a	n/a	kekdkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534607	59	n/a	n/a	ekeedkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534640	76	n/a	n/a	ekedkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534673	89	n/a	n/a	eekkkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534706	86	n/a	n/a	eekdkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534743	79	n/a	n/a	ekekdkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534778	80	n/a	n/a	keekdkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534813	83	n/a	n/a	ekkkdkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534843	82	n/a	n/a	edkdkkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534873	83	n/a	n/a	kdedkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534903	78	n/a	n/a	eekdkkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534933	83	n/a	n/a	kdkkkkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534963	70	n/a	n/a	kddedkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	534993	78	n/a	n/a	eddkkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	535023	56	n/a	n/a	eeeedkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	535056	59	n/a	n/a	eeeedkkkkkkkkkkk
9599	9614	GAAGTTTACCAAGCGG	498	535089	73	n/a	n/a	eeekkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	535122	39	n/a	n/a	eeeedkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	535155	60	n/a	n/a	ededkkkkkkkkkkke
9599	9614	GAAGTTTACCAAGCGG	498	535188	41	n/a	n/a	edkdedkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534542	75	n/a	n/a	kekedkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534575	82	n/a	n/a	kekdkkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534608	72	n/a	n/a	ekeedkkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534641	69	n/a	n/a	ekedkkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534674	84	n/a	n/a	eekkkkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534707	78	n/a	n/a	eekdkkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534744	72	n/a	n/a	ekekdkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534779	75	n/a	n/a	keekdkkkkkkkkkke
9939	9954	ACCTCTGGACACCGGG	499	534814	81	n/a	n/a	ekkkdkkkkkkkkkke

9939	9954	ACCTCTGGACACCGGG	499	534844	75	n/a	n/a	edkdddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	534874	70	n/a	n/a	kdeddddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	534904	71	n/a	n/a	eekdddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	534934	73	n/a	n/a	kddkdddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	534964	72	n/a	n/a	kddeddddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	534994	69	n/a	n/a	eddkdddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	535024	56	n/a	n/a	eeeeddddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	535057	63	n/a	n/a	eeeeddddddddddkkk
9939	9954	ACCTCTGGACACCGGG	499	535090	64	n/a	n/a	eeekdddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	535123	40	n/a	n/a	eeeeddddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	535156	47	n/a	n/a	ededkdddddddddkke
9939	9954	ACCTCTGGACACCGGG	499	535189	48	n/a	n/a	edkdeddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	534515	52	n/a	n/a	kekeddddddddddkek
11839	11854	AATGGTCAGGGCTGGT	34	534548	85	n/a	n/a	kekddddddddddekek
11839	11854	AATGGTCAGGGCTGGT	34	534581	75	n/a	n/a	ekeeddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	534614	83	n/a	n/a	ekedddddddddddekke
11839	11854	AATGGTCAGGGCTGGT	34	534647	65	n/a	n/a	eekkddddddddddeke
11839	11854	AATGGTCAGGGCTGGT	34	534680	88	n/a	n/a	eekddddddddddekeke
11839	11854	AATGGTCAGGGCTGGT	34	534717	76	n/a	n/a	ekedkdddddddddkeke
11839	11854	AATGGTCAGGGCTGGT	34	534752	79	n/a	n/a	keekdddddddddkeek
11839	11854	AATGGTCAGGGCTGGT	34	534787	90	n/a	n/a	ekkkdddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	535030	77	n/a	n/a	eeeeddddddddddkkk
11839	11854	AATGGTCAGGGCTGGT	34	535063	75	n/a	n/a	eeekdddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	535096	54	n/a	n/a	eeeeddddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	535129	66	n/a	n/a	ededkdddddddddkke
11839	11854	AATGGTCAGGGCTGGT	34	535162	49	n/a	n/a	edkdeddddddddddkke
11847	11862	GAGGAGACAATGGTCA	500	534543	66	n/a	n/a	kekeddddddddddkek
11847	11862	GAGGAGACAATGGTCA	500	534576	69	n/a	n/a	kekddddddddddekek
11847	11862	GAGGAGACAATGGTCA	500	534609	77	n/a	n/a	ekeeddddddddddkke
11847	11862	GAGGAGACAATGGTCA	500	534642	62	n/a	n/a	ekedddddddddddekke
11847	11862	GAGGAGACAATGGTCA	500	534675	80	n/a	n/a	eekkddddddddddeke
11847	11862	GAGGAGACAATGGTCA	500	534708	81	n/a	n/a	eekddddddddddekeke
11847	11862	GAGGAGACAATGGTCA	500	534745	68	n/a	n/a	ekedkdddddddddkeke
11847	11862	GAGGAGACAATGGTCA	500	534780	69	n/a	n/a	keekdddddddddkeek
11847	11862	GAGGAGACAATGGTCA	500	534815	85	n/a	n/a	ekkkdddddddddkke
11847	11862	GAGGAGACAATGGTCA	500	534845	72	n/a	n/a	edkdddddddddkdke
11847	11862	GAGGAGACAATGGTCA	500	534875	56	n/a	n/a	kdeddddddddddkke
11847	11862	GAGGAGACAATGGTCA	500	534905	65	n/a	n/a	eekdddddddddkdke
11847	11862	GAGGAGACAATGGTCA	500	534935	78	n/a	n/a	kddkdddddddddkdke
11847	11862	GAGGAGACAATGGTCA	500	534965	48	n/a	n/a	kddeddddddddddkdke
11847	11862	GAGGAGACAATGGTCA	500	534995	62	n/a	n/a	eddkdddddddddkdke
11847	11862	GAGGAGACAATGGTCA	500	535025	58	n/a	n/a	eeeeddddddddddkdke
11847	11862	GAGGAGACAATGGTCA	500	535058	60	n/a	n/a	eeeeddddddddddkkk

11847	11862	GAGGAGACAATGGTCA	500	535091	61	n/a	n/a	eeekdddddddkke
11847	11862	GAGGAGACAATGGTCA	500	535124	51	n/a	n/a	eeeeedddddddkke
11847	11862	GAGGAGACAATGGTCA	500	535157	55	n/a	n/a	ededkdddddddkke
11847	11862	GAGGAGACAATGGTCA	500	535190	47	n/a	n/a	edkdedddddddkke
12082	12097	TGGATATTCAACTGTG	501	534517	71	552	567	kekeddddddddkkek
12082	12097	TGGATATTCAACTGTG	501	534550	80	552	567	kekddddddddekek
12082	12097	TGGATATTCAACTGTG	501	534583	70	552	567	ekeeddddddddkke
12082	12097	TGGATATTCAACTGTG	501	534616	84	552	567	ekeddddddddekke
12082	12097	TGGATATTCAACTGTG	501	534649	68	552	567	eekddddddddeke
12082	12097	TGGATATTCAACTGTG	501	534682	87	552	567	eekdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534719	90	552	567	ekekdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534754	83	552	567	keekdddddddkkeek
12082	12097	TGGATATTCAACTGTG	501	534789	86	552	567	ekkdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534819	69	552	567	edkdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534849	62	552	567	kdeddddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534879	69	552	567	eekdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534909	73	552	567	kdkkdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534939	49	552	567	kddeddddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534969	47	552	567	eddkdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	534999	51	552	567	eeeeeddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	535032	51	552	567	eeeeeddddddkkkk
12082	12097	TGGATATTCAACTGTG	501	535065	64	552	567	eeekdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	535098	31	552	567	eeeeeddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	535131	31	552	567	ededkdddddddkkeke
12082	12097	TGGATATTCAACTGTG	501	535164	40	552	567	edkdeddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534518	81	568	583	kekeddddddddkkek
12098	12113	TAGGTATTTTCCACA	502	534551	88	568	583	kekddddddddekek
12098	12113	TAGGTATTTTCCACA	502	534584	78	568	583	ekeeddddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534617	80	568	583	ekeddddddddekkeke
12098	12113	TAGGTATTTTCCACA	502	534650	83	568	583	eekddddddddekeke
12098	12113	TAGGTATTTTCCACA	502	534683	93	568	583	eekdddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534720	87	568	583	ekekdddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534755	82	568	583	keekdddddddkkeek
12098	12113	TAGGTATTTTCCACA	502	534790	89	568	583	ekkdddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534820	64	568	583	edkdddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534850	38	568	583	kdeddddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534880	68	568	583	eekdddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534910	60	568	583	kdkkdddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534940	37	568	583	kddeddddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	534970	59	568	583	eddkdddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	535000	30	568	583	eeeeeddddddkkeke
12098	12113	TAGGTATTTTCCACA	502	535033	44	568	583	eeeeeddddddkkkk
12098	12113	TAGGTATTTTCCACA	502	535066	64	568	583	eeekdddddddkkeke

12098	12113	TAGGTATTTTCCACA	502	535099	22	568	583	eeeeedddddddkke
12098	12113	TAGGTATTTTCCACA	502	535132	54	568	583	ededkddddddkke
12098	12113	TAGGTATTTTCCACA	502	535165	45	568	583	edkdedddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534544	80	n/a	n/a	kekedddddddkkek
12512	12527	ATAGCTTTGATCCAAT	503	534577	83	n/a	n/a	kekdddddddekek
12512	12527	ATAGCTTTGATCCAAT	503	534610	62	n/a	n/a	ekeedddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534643	66	n/a	n/a	ekeddddddddekke
12512	12527	ATAGCTTTGATCCAAT	503	534676	95	n/a	n/a	eekkdddddddeke
12512	12527	ATAGCTTTGATCCAAT	503	534709	86	n/a	n/a	eekdddddddkeke
12512	12527	ATAGCTTTGATCCAAT	503	534746	73	n/a	n/a	ekekddddddkeke
12512	12527	ATAGCTTTGATCCAAT	503	534781	71	n/a	n/a	keekddddddkeek
12512	12527	ATAGCTTTGATCCAAT	503	534816	83	n/a	n/a	ekkdddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534846	73	n/a	n/a	edkdddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534876	39	n/a	n/a	kdeddddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534906	67	n/a	n/a	eekdddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534936	66	n/a	n/a	kddkdddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534966	48	n/a	n/a	kddedddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	534996	56	n/a	n/a	eddkdddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	535026	39	n/a	n/a	eeeedddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	535059	45	n/a	n/a	eeeedddddddkkk
12512	12527	ATAGCTTTGATCCAAT	503	535092	48	n/a	n/a	eeekdddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	535125	26	n/a	n/a	eeeeeddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	535158	44	n/a	n/a	ededkddddddkke
12512	12527	ATAGCTTTGATCCAAT	503	535191	34	n/a	n/a	edkdeddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534545	83	n/a	n/a	kekedddddddkkek
12513	12528	CATAGCTTTGATCCAA	504	534578	81	n/a	n/a	kekdddddddekek
12513	12528	CATAGCTTTGATCCAA	504	534611	78	n/a	n/a	ekeedddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534644	72	n/a	n/a	ekeddddddddekke
12513	12528	CATAGCTTTGATCCAA	504	534677	92	n/a	n/a	eekkdddddddeke
12513	12528	CATAGCTTTGATCCAA	504	534710	78	n/a	n/a	eekdddddddkeke
12513	12528	CATAGCTTTGATCCAA	504	534747	85	n/a	n/a	ekekddddddkeke
12513	12528	CATAGCTTTGATCCAA	504	534782	85	n/a	n/a	keekddddddkeek
12513	12528	CATAGCTTTGATCCAA	504	534817	88	n/a	n/a	ekkdddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534847	73	n/a	n/a	edkdddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534877	66	n/a	n/a	kdeddddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534907	73	n/a	n/a	eekdddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534937	85	n/a	n/a	kddkdddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534967	80	n/a	n/a	kddedddddddkke
12513	12528	CATAGCTTTGATCCAA	504	534997	74	n/a	n/a	eddkdddddddkke
12513	12528	CATAGCTTTGATCCAA	504	535027	64	n/a	n/a	eeeedddddddkke
12513	12528	CATAGCTTTGATCCAA	504	535060	68	n/a	n/a	eeeedddddddkkk
12513	12528	CATAGCTTTGATCCAA	504	535093	73	n/a	n/a	eeekdddddddkke
12513	12528	CATAGCTTTGATCCAA	504	535126	42	n/a	n/a	eeeeeddddddkke

12513	12528	CATAGCTTTGATCCAA	504	535159	49	n/a	n/a	ededkdddddddkke
12513	12528	CATAGCTTTGATCCAA	504	535192	51	n/a	n/a	edkdeddddddskke
14076	14091	AACATGTACTCCGTGA	505	534519	87	1133	1148	kekdeddddddskke
14076	14091	AACATGTACTCCGTGA	505	534552	85	1133	1148	kekdddddskke
14076	14091	AACATGTACTCCGTGA	505	534585	76	1133	1148	ekeddddddskke
14076	14091	AACATGTACTCCGTGA	505	534618	78	1133	1148	ekeddddddskke
14076	14091	AACATGTACTCCGTGA	505	534651	79	1133	1148	eekdddddskke
14076	14091	AACATGTACTCCGTGA	505	534684	87	1133	1148	eekdddddskke
14076	14091	AACATGTACTCCGTGA	505	534721	89	1133	1148	ekeddddddskke
14076	14091	AACATGTACTCCGTGA	505	534756	90	1133	1148	keekdddddskke
14076	14091	AACATGTACTCCGTGA	505	534791	84	1133	1148	ekkdidddddskke
14076	14091	AACATGTACTCCGTGA	505	534821	79	1133	1148	edkdddddskke
14076	14091	AACATGTACTCCGTGA	505	534851	64	1133	1148	kdeddddddskke
14076	14091	AACATGTACTCCGTGA	505	534881	65	1133	1148	eekdddddskke
14076	14091	AACATGTACTCCGTGA	505	534911	85	1133	1148	kdkdddddskke
14076	14091	AACATGTACTCCGTGA	505	534941	66	1133	1148	kddeddddskke
14076	14091	AACATGTACTCCGTGA	505	534971	75	1133	1148	eddkdddddskke
14076	14091	AACATGTACTCCGTGA	505	535001	62	1133	1148	eeeeddddskke
14076	14091	AACATGTACTCCGTGA	505	535034	65	1133	1148	eeeeddddskk
14076	14091	AACATGTACTCCGTGA	505	535067	76	1133	1148	eeekdddskke
14076	14091	AACATGTACTCCGTGA	505	535100	5	1133	1148	eeeeddddskke
14076	14091	AACATGTACTCCGTGA	505	535133	30	1133	1148	ededkdddskke
14076	14091	AACATGTACTCCGTGA	505	535166	23	1133	1148	edkdeddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534520	87	1151	1166	kekdeddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534553	79	1151	1166	kekdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534586	60	1151	1166	ekeddddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534619	62	1151	1166	ekeddddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534652	84	1151	1166	eekdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534685	84	1151	1166	eekdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534722	75	1151	1166	ekeddddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534757	81	1151	1166	keekdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534792	87	1151	1166	ekkdidddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534822	80	1151	1166	edkdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534852	38	1151	1166	kdeddddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534882	75	1151	1166	eekdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534912	74	1151	1166	kdkdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534942	58	1151	1166	kddeddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	534972	59	1151	1166	eddkdddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	535002	50	1151	1166	eeeeddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	535035	57	1151	1166	eeeeddddskk
14094	14109	TCCGAGTAGCCGGCAC	251	535068	67	1151	1166	eeekdddskke
14094	14109	TCCGAGTAGCCGGCAC	251	535101	24	1151	1166	eeeeddddskke
14094	14109	TCCGAGTAGCCGGCAC	251	535134	23	1151	1166	ededkdddskke

14094	14109	TCCGAGTAGCCGGCAC	251	535167	26	1151	1166	edkdeddddddddkke
14243	14258	TGTACTGGGAGACCCT	252	534513	90	1300	1315	kekeddddddddkkek
14243	14258	TGTACTGGGAGACCCT	252	534546	92	1300	1315	kekdddddddekek
14243	14258	TGTACTGGGAGACCCT	252	534579	78	1300	1315	ekeeddddddddkke
14243	14258	TGTACTGGGAGACCCT	252	534612	82	1300	1315	ekeddddddddekke
14243	14258	TGTACTGGGAGACCCT	252	534645	73	1300	1315	eekdddddddeke
14243	14258	TGTACTGGGAGACCCT	252	534678	91	1300	1315	eekdddddddkke
14243	14258	TGTACTGGGAGACCCT	252	534715	87	1300	1315	ekekdddddddkke
14243	14258	TGTACTGGGAGACCCT	252	534750	88	1300	1315	keekddddddkkeek
14243	14258	TGTACTGGGAGACCCT	252	534785	89	1300	1315	ekkkdddddddkke
14243	14258	TGTACTGGGAGACCCT	252	535028	52	1300	1315	eeeedddddddkkk
14243	14258	TGTACTGGGAGACCCT	252	535061	73	1300	1315	eeekdddddddkke
14243	14258	TGTACTGGGAGACCCT	252	535094	61	1300	1315	eeeeeeeeeeeekkke
14243	14258	TGTACTGGGAGACCCT	252	535127	59	1300	1315	ededkdddddddkke
14243	14258	TGTACTGGGAGACCCT	252	535160	62	1300	1315	edkdeddddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534521	86	2157	2172	kekeddddddddkkek
15100	15115	CACATGGAGTCAGCAT	506	534554	87	2157	2172	kekdddddddekek
15100	15115	CACATGGAGTCAGCAT	506	534587	62	2157	2172	ekeeddddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534620	68	2157	2172	ekeddddddddekke
15100	15115	CACATGGAGTCAGCAT	506	534653	77	2157	2172	eekkkdddddddeke
15100	15115	CACATGGAGTCAGCAT	506	534686	90	2157	2172	eekkkdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534723	88	2157	2172	ekekdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534758	79	2157	2172	keekddddddkkeek
15100	15115	CACATGGAGTCAGCAT	506	534793	85	2157	2172	ekkkdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534823	81	2157	2172	edkdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534853	59	2157	2172	kdeddddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534883	69	2157	2172	eekkkdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534913	76	2157	2172	kdkkdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534943	53	2157	2172	kddeddddddddkke
15100	15115	CACATGGAGTCAGCAT	506	534973	61	2157	2172	eddkdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	535003	53	2157	2172	eeeeeeeeeeeekkke
15100	15115	CACATGGAGTCAGCAT	506	535036	35	2157	2172	eeeeeeeeeeeekkkk
15100	15115	CACATGGAGTCAGCAT	506	535069	62	2157	2172	eeekkkkkkkdkke
15100	15115	CACATGGAGTCAGCAT	506	535102	31	2157	2172	eeeeeeeeeeeekkke
15100	15115	CACATGGAGTCAGCAT	506	535135	44	2157	2172	ededkdddddddkke
15100	15115	CACATGGAGTCAGCAT	506	535168	34	2157	2172	edkdeddddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534522	83	2186	2201	kekeddddddddkkek
15129	15144	AGCTAAACAACCGCCT	507	534555	81	2186	2201	kekdddddddekek
15129	15144	AGCTAAACAACCGCCT	507	534588	72	2186	2201	ekeeddddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534621	74	2186	2201	ekeddddddddekke
15129	15144	AGCTAAACAACCGCCT	507	534654	78	2186	2201	eekkkkkkkdeke
15129	15144	AGCTAAACAACCGCCT	507	534687	91	2186	2201	eekkkkkkkdkke
15129	15144	AGCTAAACAACCGCCT	507	534724	84	2186	2201	ekekdddddddkke

15129	15144	AGCTAAACAACCGCCT	507	534759	86	2186	2201	keekdddddddkkeek
15129	15144	AGCTAAACAACCGCCT	507	534794	78	2186	2201	ekkkdddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534824	75	2186	2201	edkkdddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534854	63	2186	2201	kdeddddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534884	60	2186	2201	eekdddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534914	75	2186	2201	kddkdddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534944	69	2186	2201	kddeddddddkke
15129	15144	AGCTAAACAACCGCCT	507	534974	66	2186	2201	eddkdddddddkke
15129	15144	AGCTAAACAACCGCCT	507	535004	56	2186	2201	eeeeddddddkke
15129	15144	AGCTAAACAACCGCCT	507	535037	50	2186	2201	eeeeddddddkkk
15129	15144	AGCTAAACAACCGCCT	507	535070	68	2186	2201	eeekdddddddkke
15129	15144	AGCTAAACAACCGCCT	507	535103	55	2186	2201	eeeeddddddkke
15129	15144	AGCTAAACAACCGCCT	507	535136	51	2186	2201	ededkdddddkke
15129	15144	AGCTAAACAACCGCCT	507	535169	54	2186	2201	edkdeddddkke
15130	15145	GAGCTAAACAACCGCC	253	534523	89	2187	2202	kekeddddddkkek
15130	15145	GAGCTAAACAACCGCC	253	534556	91	2187	2202	kekdddddddekek
15130	15145	GAGCTAAACAACCGCC	253	534589	88	2187	2202	ekeeddddddkke
15130	15145	GAGCTAAACAACCGCC	253	534622	93	2187	2202	ekeddddddekke
15130	15145	GAGCTAAACAACCGCC	253	534655	72	2187	2202	eekdddddddeke
15130	15145	GAGCTAAACAACCGCC	253	534688	92	2187	2202	eekddddddkeke
15130	15145	GAGCTAAACAACCGCC	253	534725	87	2187	2202	ekedddddkeke
15130	15145	GAGCTAAACAACCGCC	253	534760	92	2187	2202	keekdddddkkeek
15130	15145	GAGCTAAACAACCGCC	253	534795	93	2187	2202	ekkkdddddkke
15130	15145	GAGCTAAACAACCGCC	253	534825	82	2187	2202	edkkdddddkke
15130	15145	GAGCTAAACAACCGCC	253	534855	73	2187	2202	kdeddddddkke
15130	15145	GAGCTAAACAACCGCC	253	534885	82	2187	2202	eekdddddkke
15130	15145	GAGCTAAACAACCGCC	253	534915	88	2187	2202	kddkdddddkke
15130	15145	GAGCTAAACAACCGCC	253	534945	82	2187	2202	kddeddddddkke
15130	15145	GAGCTAAACAACCGCC	253	534975	68	2187	2202	eddkdddddkke
15130	15145	GAGCTAAACAACCGCC	253	535005	69	2187	2202	eeeeddddddkke
15130	15145	GAGCTAAACAACCGCC	253	535038	72	2187	2202	eeeeddddddkkk
15130	15145	GAGCTAAACAACCGCC	253	535071	74	2187	2202	eeekdddddkke
15130	15145	GAGCTAAACAACCGCC	253	535104	61	2187	2202	eeeeddddddkke
15130	15145	GAGCTAAACAACCGCC	253	535137	67	2187	2202	ededkdddddkke
15130	15145	GAGCTAAACAACCGCC	253	535170	51	2187	2202	edkdeddddkke
15131	15146	AGAGCTAAACAACCGC	254	534524	95	2188	2203	kekeddddddkkek
15131	15146	AGAGCTAAACAACCGC	254	534557	98	2188	2203	kekdddddddekek
15131	15146	AGAGCTAAACAACCGC	254	534590	91	2188	2203	ekeeddddddkke
15131	15146	AGAGCTAAACAACCGC	254	534623	91	2188	2203	ekeddddddekke
15131	15146	AGAGCTAAACAACCGC	254	534656	90	2188	2203	eekdddddddeke
15131	15146	AGAGCTAAACAACCGC	254	534689	92	2188	2203	eekddddddkeke
15131	15146	AGAGCTAAACAACCGC	254	534726	57	2188	2203	ekedddddkeke
15131	15146	AGAGCTAAACAACCGC	254	534761	89	2188	2203	keekdddddkkeek

15131	15146	AGAGCTAAACAACCGC	254	534796	93	2188	2203	ekkdddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	534826	89	2188	2203	edkdddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	534856	87	2188	2203	kdeddddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	534886	85	2188	2203	eekdddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	534916	87	2188	2203	kddkdddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	534946	86	2188	2203	kddeddddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	534976	77	2188	2203	eddkdddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	535006	83	2188	2203	eeeeddddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	535039	86	2188	2203	eeeeddddddddddkkk
15131	15146	AGAGCTAAACAACCGC	254	535072	87	2188	2203	eeekdddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	535105	68	2188	2203	eeeeddddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	535138	70	2188	2203	ededkdddddddddkke
15131	15146	AGAGCTAAACAACCGC	254	535171	65	2188	2203	edkdeddddddddddkke
15132	15147	GAGAGCTAAACAACCG	255	534558	92	2189	2204	kekddddddddddekek
15132	15147	GAGAGCTAAACAACCG	255	534591	91	2189	2204	ekeeddddddddddkke
15132	15147	GAGAGCTAAACAACCG	255	534624	86	2189	2204	ekedddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	534657	90	2189	2204	eekkddddddddddeke
15132	15147	GAGAGCTAAACAACCG	255	534690	76	2189	2204	eekddddddddddekeke
15132	15147	GAGAGCTAAACAACCG	255	534727	92	2189	2204	ekedkddddddddddekeke
15132	15147	GAGAGCTAAACAACCG	255	534762	91	2189	2204	keekdddddddddkeek
15132	15147	GAGAGCTAAACAACCG	255	534797	94	2189	2204	ekkddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	534827	90	2189	2204	edkddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	534857	80	2189	2204	kdedddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	534887	76	2189	2204	eekddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	534917	91	2189	2204	kddkddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	534947	91	2189	2204	kddedddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	534977	86	2189	2204	eddkddddddddddekke
15132	15147	GAGAGCTAAACAACCG	255	535007	80	2189	2204	eeeeddddddddddkke
15132	15147	GAGAGCTAAACAACCG	255	535040	86	2189	2204	eeeeddddddddddkkk
15132	15147	GAGAGCTAAACAACCG	255	535073	87	2189	2204	eeekdddddddddkke
15132	15147	GAGAGCTAAACAACCG	255	535106	70	2189	2204	eeeeddddddddddkke
15132	15147	GAGAGCTAAACAACCG	255	535139	73	2189	2204	ededkdddddddddkke
15132	15147	GAGAGCTAAACAACCG	255	535172	69	2189	2204	edkdedddddddddddekke
15164	15179	TGAAGATGATAATGGA	61	534514	90	2221	2236	kekeddddddddddkek
15164	15179	TGAAGATGATAATGGA	61	534547	92	2221	2236	kekddddddddddekek
15164	15179	TGAAGATGATAATGGA	61	534580	78	2221	2236	ekeeddddddddddkke
15164	15179	TGAAGATGATAATGGA	61	534613	80	2221	2236	ekedddddddddddekke
15164	15179	TGAAGATGATAATGGA	61	534646	79	2221	2236	eekkddddddddddeke
15164	15179	TGAAGATGATAATGGA	61	534679	93	2221	2236	eekddddddddddekeke
15164	15179	TGAAGATGATAATGGA	61	534716	94	2221	2236	ekekddddddddddekeke
15164	15179	TGAAGATGATAATGGA	61	534751	86	2221	2236	keekdddddddddkeek
15164	15179	TGAAGATGATAATGGA	61	534786	83	2221	2236	ekkddddddddddekke
15164	15179	TGAAGATGATAATGGA	61	535029	45	2221	2236	eeeeddddddddddkkk

15164	15179	TGAAGATGATAATGGA	61	535062	81	2221	2236	eeekdddddddkke
15164	15179	TGAAGATGATAATGGA	61	535095	57	2221	2236	eeeeeeeeeeekke
15164	15179	TGAAGATGATAATGGA	61	535128	58	2221	2236	ededeeeeeeekke
15164	15179	TGAAGATGATAATGGA	61	535161	49	2221	2236	edkedeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534526	94	2240	2255	kekedeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534559	95	2240	2255	kekdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534592	93	2240	2255	ekedeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534625	93	2240	2255	ekedeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534658	93	2240	2255	eekdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534691	96	2240	2255	eekdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534728	93	2240	2255	eekdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534763	93	2240	2255	keekdeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534798	97	2240	2255	ekkdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534828	94	2240	2255	edkdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534858	92	2240	2255	kddeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534888	93	2240	2255	eekdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534918	95	2240	2255	kdkdeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534948	93	2240	2255	kdddeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	534978	91	2240	2255	eddkdeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	535008	88	2240	2255	eeedeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	535041	87	2240	2255	eeedeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	535074	90	2240	2255	eeekdeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	535107	78	2240	2255	eeedeeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	535140	81	2240	2255	ededeeeeeeekke
15183	15198	GCTTCTGAATTGTCTG	256	535173	81	2240	2255	edkedeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534527	95	2243	2258	kekedeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534560	96	2243	2258	kekdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534593	87	2243	2258	ekedeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534626	85	2243	2258	ekedeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534659	90	2243	2258	eekdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534692	91	2243	2258	eekdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534729	91	2243	2258	eekdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534764	91	2243	2258	keekdeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534799	96	2243	2258	ekkdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534829	91	2243	2258	edkdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534859	87	2243	2258	kddeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534889	81	2243	2258	eekdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534919	92	2243	2258	kdkdeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534949	91	2243	2258	kdddeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	534979	84	2243	2258	eddkdeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	535009	78	2243	2258	eeedeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	535042	76	2243	2258	eeedeeeeeeeekke
15186	15201	GATGCTTCTGAATTGT	258	535075	83	2243	2258	eeekdeeeeeekke

15186	15201	GATGCTTCTGAATTGT	258	535108	64	2243	2258	eeeeeddddddddkke
15186	15201	GATGCTTCTGAATTGT	258	535141	69	2243	2258	ededkdddddddkke
15186	15201	GATGCTTCTGAATTGT	258	535174	65	2243	2258	edkdeddddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534528	94	2250	2265	kekeddddddddkkek
15193	15208	GCATGGTGATGCTTCT	260	534561	0	2250	2265	kekddddddddekek
15193	15208	GCATGGTGATGCTTCT	260	534594	92	2250	2265	ekeeddddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534627	90	2250	2265	ekeddddddddekke
15193	15208	GCATGGTGATGCTTCT	260	534660	92	2250	2265	eekddddddddeke
15193	15208	GCATGGTGATGCTTCT	260	534693	95	2250	2265	eekdddddddkkeke
15193	15208	GCATGGTGATGCTTCT	260	534730	93	2250	2265	ekeddddddddkkeke
15193	15208	GCATGGTGATGCTTCT	260	534765	92	2250	2265	keekdddddddkkeek
15193	15208	GCATGGTGATGCTTCT	260	534800	93	2250	2265	ekkdddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534830	93	2250	2265	edkdddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534860	85	2250	2265	kdeddddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534890	91	2250	2265	eekdddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534920	93	2250	2265	kddkdddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534950	90	2250	2265	kddeddddddkke
15193	15208	GCATGGTGATGCTTCT	260	534980	88	2250	2265	eddkdddddddkke
15193	15208	GCATGGTGATGCTTCT	260	535010	88	2250	2265	eeeeddddddkke
15193	15208	GCATGGTGATGCTTCT	260	535043	89	2250	2265	eeeddddddddkkk
15193	15208	GCATGGTGATGCTTCT	260	535076	88	2250	2265	eeekdddddddkke
15193	15208	GCATGGTGATGCTTCT	260	535109	76	2250	2265	eeeeeddddddkke
15193	15208	GCATGGTGATGCTTCT	260	535142	86	2250	2265	ededkdddddkke
15193	15208	GCATGGTGATGCTTCT	260	535175	71	2250	2265	edkdeddddddkke
15196	15211	CATGCATGGTGATGCT	261	534529	70	2253	2268	kekedddddddkek
15196	15211	CATGCATGGTGATGCT	261	534562	86	2253	2268	kekdddddddekek
15196	15211	CATGCATGGTGATGCT	261	534595	56	2253	2268	ekeeddddddkke
15196	15211	CATGCATGGTGATGCT	261	534628	73	2253	2268	ekeddddddddekke
15196	15211	CATGCATGGTGATGCT	261	534661	64	2253	2268	eekdddddddeke
15196	15211	CATGCATGGTGATGCT	261	534694	75	2253	2268	eekdddddddkkeke
15196	15211	CATGCATGGTGATGCT	261	534731	47	2253	2268	ekedddddddkeke
15196	15211	CATGCATGGTGATGCT	261	534766	30	2253	2268	keekddddddkeek
15196	15211	CATGCATGGTGATGCT	261	534801	83	2253	2268	ekkdddddddkke
15196	15211	CATGCATGGTGATGCT	261	534831	84	2253	2268	edkdddddddkke
15196	15211	CATGCATGGTGATGCT	261	534861	71	2253	2268	kdeddddddddkke
15196	15211	CATGCATGGTGATGCT	261	534891	73	2253	2268	eekdddddddkke
15196	15211	CATGCATGGTGATGCT	261	534921	55	2253	2268	kddkdddddddkke
15196	15211	CATGCATGGTGATGCT	261	534951	61	2253	2268	kddeddddddkke
15196	15211	CATGCATGGTGATGCT	261	534981	48	2253	2268	eddkdddddddkke
15196	15211	CATGCATGGTGATGCT	261	535011	54	2253	2268	eeeeddddddkke
15196	15211	CATGCATGGTGATGCT	261	535044	46	2253	2268	eeeddddddddkkk
15196	15211	CATGCATGGTGATGCT	261	535077	29	2253	2268	eeekdddddddkke
15196	15211	CATGCATGGTGATGCT	261	535110	19	2253	2269	eeeeeddddddkke

15196	15211	CATGCATGGTGATGCT	261	535143	15	2253	2268	ededkdddddddkke
15196	15211	CATGCATGGTGATGCT	261	535176	37	2253	2268	edkdeddddddddkke

Example 22: Modified antisense oligonucleotides comprising cEt and MOE modifications targeting intronic repeat sequences of the human coagulation Factor VII genomic sequence

Additional antisense oligonucleotides were designed targeting intronic repeat regions of SEQ ID NO: 1. The newly designed modified antisense oligonucleotides and their motifs are described in Table 23. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The ‘Sugar Chemistry’ column provides the sugar modifications throughout each oligonucleotide: ‘d’ indicates a 2’-deoxynucleoside, ‘k’ indicates a constrained ethyl (cEt) nucleoside, and ‘e’ indicates a 2’-O-methoxyethyl nucleoside. The ‘Sequence’ column provides the nucleobase sequence for each SEQ ID NO.

Each oligonucleotide listed in Table 23 is targeted to intronic regions of human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000). “Start site” indicates the 5’-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. “Stop site” indicates the 3’-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. Oligonucleotides having multiple start and stop sites target a region that is repeated within a Factor VII sequence (e.g., within SEQ ID NO: 1).

Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2927 was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

Table 23

Percent inhibition of human Factor VII mRNA levels by modified antisense oligonucleotides targeted to SEQ ID NO: 1

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	% inhibition	Sugar Chemistry
15263	15276	TGTGCAGCCCGGCA	508	472998	90	kkdddddddddkk
6642	6657	GAGGTGACCCGTGAGC	30	473327	88	kkkddddddddddeee
6712	6727	GTGTGAGGTGACCTGT	509	537024	74	eeeddddddddddkkk
6834	6849					
7022	7037					
7140	7155					
7397	7412					
7463	7478					
7862	7877					

6713	6728	AGTGTGAGGTGACCTG	510	537025	79	eeedddddddddkkk
6835	6850					
7398	7413					
7863	7878					
6714	6729	GAGTGTGAGGTGACCT	511	537026	76	eeedddddddddkkk
6836	6851					
7399	7414					
7864	7879					
6716	6731	GTGAGTGTGAGGTGAC	512	537028	37	eeedddddddddkkk
6838	6853					
6960	6975					
7078	7093					
7196	7211					
7401	7416					
7866	7881					
6717	6732	TGTGAGTGTGAGGTGA	513	537029	45	eeedddddddddkkk
6839	6854					
6961	6976					
7079	7094					
7197	7212					
7338	7353					
7402	7417					
7867	7882					
6718	6733	CTGTGAGTGTGAGGTG	514	537030	67	eeedddddddddkkk
6736	6751					
6840	6855					
6858	6873					
6962	6977					
7080	7095					
7198	7213					
7339	7354					
7403	7418					
7637	7652					
7868	7883					
8146	8161					
8393	8408					
6719	6734	CCTGTGAGTGTGAGGT	515	537031	59	eeedddddddddkkk
6737	6752					
6841	6856					
6859	6874					
6963	6978					
7081	7096					
7199	7214					
7340	7355					
7404	7419					
7638	7653					

7869	7884					
8147	8162					
8394	8409					
6720	6735	TCCTGTGAGTGTGAGG	516	537032	9	eeedddddddddkkk
6842	6857					
6964	6979					
7082	7097					
7200	7215					
7341	7356					
7405	7420					
7870	7885					
8395	8410					
8485	8500					
6721	6736	GTCCTGTGAGTGTGAG	517	537033	65	eeedddddddddkkk
6843	6858					
6965	6980					
7083	7098					
7201	7216					
7342	7357					
7406	7421					
7871	7886					
6722	6737	TGCCTGTGAGTGTGA	518	537034	71	eeedddddddddkkk
6844	6859					
6966	6981					
7084	7099					
7202	7217					
7343	7358					
7407	7422					
7872	7887					
6723	6738	GTGTCCTGTGAGTGTG	519	537035	68	eeedddddddddkkk
6845	6860					
6967	6982					
7085	7100					
7203	7218					
7344	7359					
7408	7423					
7873	7888					
6724	6739	GGTGTCTGTGAGTGT	520	537036	74	eeedddddddddkkk
6846	6861					
6968	6983					
7086	7101					
7204	7219					
7345	7360					
7409	7424					
7874	7889					
6726	6741	GAGGTGTCCTGTGAGT	521	537038	69	eeedddddddddkkk

6848	6863					
6970	6985					
7088	7103					
7206	7221					
7347	7362					
7411	7426					
7876	7891					
6727	6742	TGAGGTGTCCTGTGAG	522	537039	67	eeddddddddddkkk
6849	6864					
6971	6986					
7089	7104					
7207	7222					
7348	7363					
7412	7427					
7628	7643					
7664	7679					
7682	7697					
7877	7892					
8137	8152					
8173	8188					
6728	6743	GTGAGGTGTCCTGTGA	523	537040	68	eeddddddddddkkk
6850	6865					
6972	6987					
7090	7105					
7208	7223					
7349	7364					
7413	7428					
7629	7644					
7665	7680					
7683	7698					
7878	7893					
8138	8153					
8174	8189					
6729	6744	TGTGAGGTGTCCTGTG	524	537041	76	eeddddddddddkkk
6851	6866					
6973	6988					
7039	7054					
7091	7106					
7157	7172					
7209	7224					
7263	7278					
7291	7306					
7350	7365					
7414	7429					
7480	7495					
7512	7527					

7526	7541					
7558	7573					
7630	7645					
7684	7699					
7879	7894					
7911	7926					
7975	7990					
8035	8050					
8067	8082					
8139	8154					
8175	8190					
6730	6745	GTGTGAGGTGTCCTGT	525	537042	77	eeedddddddddkkk
6852	6867					
6974	6989					
7040	7055					
7092	7107					
7158	7173					
7210	7225					
7292	7307					
7351	7366					
7513	7528					
7559	7574					
7631	7646					
7685	7700					
8068	8083					
8140	8155					
8176	8191					
6731	6746	AGTGTGAGGTGTCCTG	526	537043	70	eeedddddddddkkk
6853	6868					
7211	7226					
7293	7308					
7560	7575					
7632	7647					
8069	8084					
8141	8156					
6732	6747	GAGTGTGAGGTGTCCT	527	537044	82	eeedddddddddkkk
6854	6869					
7212	7227					
7294	7309					
7561	7576					
7633	7648					
8070	8085					
8142	8157					
6733	6748	TGAGTGTGAGGTGTCC	528	537045	69	eeedddddddddkkk
6855	6870					
7213	7228					

7295	7310					
7562	7577					
7634	7649					
8071	8086					
8143	8158					
6735	6750	TGTGAGTGTGAGGTGT	529	537047	35	eeedddddddddkkk
6857	6872					
7636	7651					
8145	8160					
8392	8407					
6739	6754	GCCCTGTGAGTGTGAG	530	537049	62	eeedddddddddkkk
6861	6876					
6741	6756	GTGCCCTGTGAGTGTG	531	537051	62	eeedddddddddkkk
6863	6878					
6755	6770	CGTGAGTGTGAAGTGT	532	537055	16	eeedddddddddkkk
6877	6892					
6943	6958					
7061	7076					
7179	7194					
6756	6771	CCGTGAGTGTGAAGTG	533	537056	25	eeedddddddddkkk
6878	6893					
6944	6959					
7062	7077					
7180	7195					
7234	7249	CCCGTGAGTGTGAAGT	534	537057	49	eeedddddddddkkk
6757	6772					
6879	6894					
6945	6960					
7063	7078					
7181	7196	ACCCGTGAGTGTGAAG	535	537058	49	eeedddddddddkkk
7235	7250					
6758	6773					
6880	6895					
6946	6961					
7064	7079	GACCCGTGAGTGTGAA	536	537059	53	eeedddddddddkkk
7182	7197					
7236	7251					
6759	6774					
6881	6896					
6947	6962	TGACCCGTGAGTGTGA	537	537060	73	eeedddddddddkkk
7065	7080					
7183	7198					
7237	7252					
6760	6775					
6882	6897					
6948	6963					

7066	7081					
7184	7199					
7238	7253					
6761	6776	GTGACCCGTGAGTGTG	538	537061	70	eeedddddddddkkk
6883	6898					
6949	6964					
7067	7082					
7185	7200					
7239	7254					
6762	6777	GGTGACCCGTGAGTGT	539	537062	69	eeedddddddddkkk
6884	6899					
6950	6965					
7068	7083					
7186	7201					
7240	7255					
6763	6778	AGGTGACCCGTGAGTG	540	537063	68	eeedddddddddkkk
6885	6900					
6951	6966					
7069	7084					
7187	7202					
7241	7256					
6764	6779	GAGGTGACCCGTGAGT	541	537064	71	eeedddddddddkkk
6886	6901					
6952	6967					
7070	7085					
7188	7203					
7242	7257					
6643	6658	TGAGGTGACCCGTGAG	542	537065	67	eeedddddddddkkk
6765	6780					
6887	6902					
6953	6968					
7071	7086					
7189	7204					
7243	7258	GTGAGGTGACCCGTGA	543	537066	68	eeedddddddddkkk
6644	6659					
6766	6781					
6888	6903					
6954	6969					
7072	7087					
7190	7205	TGTGAGGTGACCCGTG	544	537067	71	eeedddddddddkkk
7244	7259					
6645	6660					
6767	6782					
6889	6904					
6955	6970					
7073	7088					

7191	7206					
7245	7260					
6646	6661	GTGTGAGGTGACCCGT	545	537068	86	eeedddddddddkkk
6768	6783					
6890	6905					
6956	6971					
7074	7089					
7192	7207					
7246	7261					
6647	6662	AGTGTGAGGTGACCCG	546	537069	82	eeedddddddddkkk
6769	6784					
6891	6906					
6957	6972					
7075	7090					
7193	7208					
6648	6663	GAGTGTGAGGTGACCC	547	537070	87	eeedddddddddkkk
6770	6785					
6892	6907					
6958	6973					
7076	7091					
7194	7209					
6697	6712	TGAGTGTGAAGTGTGC	548	537792	36	eeedddddddddkkk
6753	6768					
6819	6834					
6875	6890					
6941	6956					
7007	7022					
7059	7074					
7125	7140					
7177	7192					
7382	7397					
7448	7463					
7795	7810					
7945	7960					
8286	8301					
6698	6713	GTGAGTGTGAAGTGTG	549	537793	35	eeedddddddddkkk
6754	6769					
6820	6835					
6876	6891					
6942	6957					
7008	7023					
7060	7075					
7126	7141					
7178	7193					
7383	7398					
7449	7464					

7796	7811					
8287	8302					
6699	6714	TGTGAGTGTGAAGTGT	550	537794	35	eeedddddddddkkk
6821	6836					
7009	7024					
7127	7142					
7384	7399					
7450	7465					
7797	7812					
8288	8303					
6700	6715	CTGTGAGTGTGAAGTG	551	537795	33	eeedddddddddkkk
6822	6837					
7010	7025					
7128	7143					
7385	7400					
7451	7466					
7798	7813					
8289	8304					
6701	6716	CCTGTGAGTGTGAAGT	552	537796	49	eeedddddddddkkk
6823	6838					
7011	7026					
7129	7144					
7386	7401					
7452	7467					
7799	7814					
8290	8305					
6702	6717	ACCTGTGAGTGTGAAG	553	537797	54	eeedddddddddkkk
6824	6839					
7012	7027					
7130	7145					
7387	7402					
7453	7468					
7800	7815					
8291	8306					
6703	6718	GACCTGTGAGTGTGAA	554	537798	68	eeedddddddddkkk
6825	6840					
7013	7028					
7131	7146					
7388	7403					
7454	7469					
7801	7816					
8292	8307					
6704	6719	TGACCTGTGAGTGTGA	555	537799	72	eeedddddddddkkk
6826	6841					
7014	7029					
7132	7147					

7389	7404					
7455	7470					
7605	7620					
7641	7656					
7802	7817					
8114	8129					
8150	8165					
8293	8308					
6705	6720	GTGACCTGTGAGTGTG	556	537800	69	eeedddddddddkkk
6827	6842					
7015	7030					
7133	7148					
7390	7405					
7456	7471					
7606	7621					
7642	7657					
7803	7818					
8115	8130					
8151	8166					
8294	8309					
6706	6721	GGTGACCTGTGAGTGT	557	537801	82	eeedddddddddkkk
6828	6843					
7016	7031					
7134	7149					
7391	7406					
7457	7472					
7607	7622					
7643	7658					
8116	8131					
8152	8167					
6707	6722	AGGTGACCTGTGAGTG	558	537802	72	eeedddddddddkkk
6829	6844					
7017	7032					
7135	7150					
7392	7407					
7458	7473					
7608	7623					
7644	7659					
8117	8132					
8153	8168					
6708	6723	GAGGTGACCTGTGAGT	559	537803	72	eeedddddddddkkk
6830	6845					
7018	7033					
7136	7151					
7393	7408					
7459	7474					

6709	6724	TGAGGTGACCTGTGAG	560	537804	67	eeedddddddddkkk
6831	6846					
7019	7034					
7137	7152					
7394	7409					
7460	7475					
7859	7874					
6710	6725	GTGAGGTGACCTGTGA	561	537805	74	eeedddddddddkkk
6832	6847					
7020	7035					
7138	7153					
7395	7410					
7461	7476					
7860	7875					
6711	6726	TGTGAGGTGACCTGTG	562	537806	70	eeedddddddddkkk
6833	6848					
7021	7036					
7139	7154					
7396	7411					
7462	7477					
7861	7876					
6691	6706	TGAAGTGTGCCCTGTG	563	537809	60	eeedddddddddkkk
6747	6762					
6813	6828					
6869	6884					
6935	6950					
7053	7068					
7171	7186					
7698	7713					
8189	8204					
6692	6707	GTGAAGTGTGCCCTGT	564	537810	71	eeedddddddddkkk
6748	6763					
6814	6829					
6870	6885					
6936	6951					
7054	7069					
7172	7187					
7699	7714					
8190	8205					
6693	6708	TGTGAAGTGTGCCCTG	565	537811	69	eeedddddddddkkk
6749	6764					
6815	6830					
6871	6886					
6937	6952					
7055	7070					
7173	7188					

7791	7806					
8282	8297					
6694	6709	GTGTGAAGTGTGCCCT	566	537812	80	eeedddddddddkkk
6750	6765					
6816	6831					
6872	6887					
6938	6953					
7056	7071					
7174	7189					
7792	7807					
8283	8298					
6695	6710	AGTGTGAAGTGTGCCC	567	537813	74	eeedddddddddkkk
6751	6766					
6817	6832					
6873	6888					
6939	6954					
7005	7020					
7057	7072					
7123	7138					
7175	7190					
7380	7395					
7446	7461					
7793	7808					
7943	7958					
8284	8299					
6696	6711	GAGTGTGAAGTGTGCC	568	537814	54	eeedddddddddkkk
6752	6767					
6818	6833					
6874	6889					
6940	6955					
7006	7021					
7058	7073					
7124	7139					
7176	7191					
7381	7396					
7447	7462					
7794	7809					
7944	7959					
8285	8300					
6678	6693	GTGTGAGGTGTCCTCT	569	537837	70	eeedddddddddkkk
6800	6815					
6922	6937					
6679	6694	TGTGTGAGGTGTCCTC	570	537838	76	eeedddddddddkkk
6801	6816					
6923	6938					
6680	6695	CTGTGTGAGGTGTCCT	571	537839	76	eeedddddddddkkk

6802	6817					
6924	6939					
7042	7057					
7160	7175					
7515	7530					
7687	7702					
8178	8193					
6681	6696	CCTGTGTGAGGTGTCC	572	537840	80	eeedddddddddkkk
6803	6818					
6925	6940					
7043	7058					
7161	7176					
7516	7531					
7688	7703					
8179	8194					
6682	6697	CCCTGTGTGAGGTGTC	573	537841	81	eeedddddddddkkk
6804	6819					
6926	6941					
7044	7059					
7162	7177					
7689	7704					
8180	8195					
6683	6698	GCCCTGTGTGAGGTGT	574	537842	75	eeedddddddddkkk
6805	6820					
6927	6942					
7045	7060					
7163	7178					
7690	7705					
8181	8196					
6684	6699	TGCCCTGTGTGAGGTG	575	537843	70	eeedddddddddkkk
6806	6821					
6928	6943					
7046	7061					
7164	7179					
7691	7706					
8182	8197					
6685	6700	GTGCCCTGTGTGAGGT	576	537844	73	eeedddddddddkkk
6807	6822					
6929	6944					
7047	7062					
7165	7180					
7692	7707					
8183	8198					
6686	6701	TGTGCCCTGTGTGAGG	577	537845	59	eeedddddddddkkk
6808	6823					
6930	6945					

7048	7063					
7166	7181					
7693	7708					
8184	8199					
6687	6702	GTGTGCCCTGTGTGAG	578	537846	51	eeedddddddddkkk
6809	6824					
6931	6946					
7049	7064					
7167	7182					
7694	7709					
8185	8200					
6688	6703	AGTGTGCCCTGTGTGA	579	537847	52	eeedddddddddkkk
6810	6825					
6932	6947					
7050	7065					
7168	7183					
7695	7710					
8186	8201					
6689	6704	AAGTGTGCCCTGTGTG	580	537848	41	eeedddddddddkkk
6811	6826					
6933	6948					
7051	7066					
7169	7184					
7696	7711					
8187	8202					
6690	6705	GAAGTGTGCCCTGTGT	581	537849	44	eeedddddddddkkk
6812	6827					
6934	6949					
7052	7067					
7170	7185					
7697	7712					
8188	8203					
6975	6990	TGTGTGAGGTGTCCTG	582	538160	69	eeedddddddddkkk
7041	7056					
7093	7108					
7159	7174					
7352	7367					
7514	7529					
7686	7701					
8177	8192					
6987	7002	TGTGAGGTGTCTTGTG	583	538172	24	eeedddddddddkkk
7105	7120					
8443	8458					
6988	7003	GTGTGAGGTGTCTTGT	584	538173	23	eeedddddddddkkk
7106	7121					
8444	8459					

7000	7015	GAAGTGTGCCCCGTGT	585	538185	68	eeedddddddddkkk
7118	7133					
7375	7390					
7441	7456					
7938	7953					
7002	7017	GTGAAGTGTGCCCCGT	585	538187	69	eeedddddddddkkk
7120	7135					
7377	7392					
7443	7458					
7940	7955					
7004	7019	GTGTGAAGTGTGCCCC	587	538189	81	eeedddddddddkkk
7122	7137					
7379	7394					
7445	7460					
7942	7957					
7024	7039	GGGTGTGAGGTGACCT	588	538191	66	eeedddddddddkkk
7142	7157					
7465	7480					
7497	7512					
7543	7558					
7896	7911					
7960	7975					
7992	8007					
8052	8067	TGGGTGTGAGGTGACC	589	538192	59	eeedddddddddkkk
7025	7040					
7143	7158					
7249	7264					
7466	7481					
7498	7513					
7544	7559					
7897	7912					
7961	7976					
7993	8008					
8053	8068	GTGGGTGTGAGGTGAC	590	538193	16	eeedddddddddkkk
7026	7041					
7144	7159					
7250	7265					
7467	7482					
7499	7514					
7545	7560					
7898	7913					
7962	7977					
7994	8009					
8054	8069	TGTGGGTGTGAGGTGA	591	538194	10	eeedddddddddkkk
7027	7042					
7145	7160					

7251	7266					
7468	7483					
7500	7515					
7546	7561					
7899	7914					
7963	7978					
7995	8010					
8055	8070					
7028	7043	CTGTGGGTGTGAGGTG	592	538195	15	eeedddddddddkkk
7146	7161					
7252	7267					
7469	7484					
7501	7516					
7547	7562					
7900	7915					
7964	7979					
7996	8011	CCTGTGGGTGTGAGGT	593	538196	3	eeedddddddddkkk
8056	8071					
7029	7044					
7147	7162					
7253	7268					
7470	7485					
7502	7517					
7548	7563					
7901	7916	TCCTGTGGGTGTGAGG	594	538197	36	eeedddddddddkkk
7965	7980					
7997	8012					
8057	8072					
7030	7045					
7148	7163					
7254	7269					
7471	7486					
7503	7518	GTCCTGTGGGTGTGAG	595	538198	49	eeedddddddddkkk
7549	7564					
7902	7917					
7966	7981					
7998	8013					
8058	8073					
7031	7046					
7149	7164					
7255	7270					
7472	7487					
7504	7519					
7550	7565					
7903	7918					
7967	7982					

7999	8014					
8059	8074					
7032	7047	TGTCTGTGGGTGTGA	596	538199	47	eeedddddddddkkk
7150	7165					
7256	7271					
7473	7488					
7505	7520					
7551	7566					
7904	7919					
7968	7983					
8000	8015					
8060	8075					
7033	7048	GTGTCTGTGGGTGTG	597	538200	57	eeedddddddddkkk
7151	7166					
7257	7272					
7474	7489					
7506	7521					
7552	7567					
7905	7920					
7969	7984					
8061	8076					
7034	7049	GGTGTCTGTGGGTGT	598	538201	71	eeedddddddddkkk
7152	7167					
7258	7273					
7475	7490					
7507	7522					
7553	7568					
7906	7921					
7970	7985					
8062	8077					
7035	7050	AGGTGTCTGTGGGTG	599	538202	60	eeedddddddddkkk
7153	7168					
7259	7274					
7476	7491					
7508	7523					
7554	7569					
7907	7922					
7971	7986					
8063	8078					
7036	7051	GAGGTGTCTGTGGGT	600	538203	55	eeedddddddddkkk
7154	7169					
7260	7275					
7477	7492					
7509	7524					
7555	7570					
7908	7923					

7972	798762					
8064	8079					
7037	7052	TGAGGTGTCCTGTGGG	601	538204	62	eedddddddddkkk
7155	7170					
7261	7276					
7478	7493					
7510	7525					
7556	7571					
7909	7924					
7973	7988					
8065	8080					
7038	7053	GTGAGGTGTCCTGTGG	602	538205	68	eedddddddddkkk
7156	7171					
7262	7277					
7479	7494					
7511	7526					
7557	7572					
7910	7925					
7974	7989					
8066	8081					
7264	7279	CTGTGAGGTGTCCTGT	603	538228	63	eedddddddddkkk
7415	7430					
7481	7496					
7527	7542					
7880	7895					
7912	7927					
7976	7991					
7265	7280	TCTGTGAGGTGTCCTG	604	538229	26	eedddddddddkkk
7416	7431					
7482	7497					
7528	7543					
7881	7896					
7913	7928					
7977	7992					
7266	7281	CTCTGTGAGGTGTCCT	605	538230	75	eedddddddddkkk
7417	7432					
7483	7498					
7529	7544					
7882	7897					
7914	7929					
7978	7993					
7267	7282	CCTCTGTGAGGTGTCC	606	538231	75	eedddddddddkkk
7418	7433					
7484	7499					
7530	7545					
7883	7898					

7915	7930					
7979	7994					
7269	7284	GACCTCTGTGAGGTGT	607	538233	52	eeedddddddddkkk
7420	7435					
7486	7501					
7532	7547					
7885	7900					
7917	7932					
7981	7996					
7271	7286	GTGACCTCTGTGAGGT	608	538235	26	eeedddddddddkkk
7422	7437					
7488	7503					
7534	7549					
7887	7902					
7919	7934					
7983	7998					
7273	7288	AGGTGACCTCTGTGAG	609	538237	28	eeedddddddddkkk
7424	7439					
7490	7505					
7536	7551					
7889	7904					
7921	7936					
7985	8000					
8017	8032					
7275	7290	TGAGGTGACCTCTGTG	610	538239	54	eeedddddddddkkk
7426	7441					
7492	7507					
7538	7553					
7891	7906					
7923	7938					
7987	8002					
8019	8034					
7277	7292	TGTGAGGTGACCTCTG	611	538241	73	eeedddddddddkkk
7428	7443					
7494	7509					
7540	7555					
7893	7908					
7925	7940					
7989	8004					
8021	8036					
7278	7293	GTGTGAGGTGACCTCT	612	538242	68	eeedddddddddkkk
7429	7444					
7495	7510					
7541	7556					
7894	7909					
7926	7941					

7990	8005					
8022	8037					
7279	7294	TGTGTGAGGTGACCTC	613	538243	61	eeedddddddddkkk
8023	8038					
7281	7296	CCTGTGTGAGGTGACC	614	538245	75	eeedddddddddkkk
8025	8040					
7289	7304	TGAGGTGTCCTGTGTG	615	538253	37	eeedddddddddkkk
7524	7539					
8033	8048					
7290	7305	GTGAGGTGTCCTGTGT	616	538254	45	eeedddddddddkkk
7525	7540					
8034	8049					
7604	7619					
7640	7655	GACCTGTGAGTGTGAG	617	538361	56	eeedddddddddkkk
8113	8128					
8149	8164					
8373	8388					
7625	7640					
7661	7676	GGTGTCTGTGAGAGT	618	538378	70	eeedddddddddkkk
7679	7694					
8134	8149					
8170	8185					
7627	7642					
7663	7678	GAGGTGTCCTGTGAGA	619	538380	68	eeedddddddddkkk
7681	7696					
7840	7855					
8136	8151					
8172	8187					
8331	8346					
7639	7654	ACCTGTGAGTGTGAGG	620	538381	57	eeedddddddddkkk
8148	8163					
2560	2575					
3257	3272					
3700	3715	CGGGACACCCACACCC	621	540361	71	eeedddddddddkkk
3717	3732					
4023	4038					
4109	4124					
4296	4311					
4551	4566					
2562	2577					
2579	2594	CCCGGGACACCCACAC	622	540362	73	eeedddddddddkkk
2613	2628					
2647	2662					
2715	2730					
2783	2798					
2817	2832					

2885	2900					
2953	2968					
3021	3036					
3055	3070					
3089	3104					
3259	3274					
3361	3376					
3565	3580					
3685	3700					
3702	3717					
3719	3734					
3736	3751					
3872	3887					
3940	3955					
4025	4040					
4111	4126					
4145	4160					
4298	4313					
4332	4347					
4434	4449					
4468	4483					
4553	4568					
2564	2579	CTCCCGGGACACCCAC	623	540363	78	eeeddddddddkkk
2632	2647					
2666	2681					
2734	2749					
2802	2817					
2836	2851					
2904	2919					
2972	2987					
3006	3021					
3040	3055					
3074	3089					
3091	3106					
3278	3293					
3380	3395					
3482	3497					
3602	3617					
3721	3736					
3755	3770					
3857	3872					
3891	3906					
3959	3974					
4045	4060					
4130	4145					
4164	4179					

4266	4281					
4317	4332					
4453	4468					
4573	4588					
2565	2580	ACTCCCGGGACACCCA	624	540364	89	eeeddddddddkkk
2633	2648					
2667	2682					
2735	2750					
2803	2818					
2837	2852					
2905	2920					
3007	3022					
3041	3056					
3075	3090					
3092	3107					
3279	3294					
3381	3396					
3483	3498					
3603	3618					
3722	3737					
3756	3771					
3858	3873					
3892	3907					
3960	3975					
4046	4061					
4131	4146					
4165	4180					
4318	4333					
4454	4469					
2566	2581	CACTCCCGGGACACCC	625	540365	83	eeeddddddddkkk
2634	2649					
2668	2683					
2702	2717					
2736	2751					
2770	2785					
2804	2819					
2838	2853					
2872	2887					
2906	2921					
2940	2955					
3008	3023					
3042	3057					
3076	3091					
3093	3108					
3127	3142					
3280	3295					

3314	3329					
3348	3363					
3382	3397					
3416	3431					
3450	3465					
3484	3499					
3518	3533					
3552	3567					
3604	3619					
3638	3653					
3672	3687					
3723	3738					
3757	3772					
3859	3874					
3893	3908					
3961	3976					
4047	4062					
4081	4096					
4132	4147					
4166	4181					
4200	4215					
4319	4334					
4387	4402					
4421	4436					
4455	4470					
2567	2582	ACACTCCCGGGACACC	626	540366	84	eeddddddddddkkk
2635	2650					
2669	2684					
2703	2718					
2737	2752					
2771	2786					
2805	2820					
2839	2854					
2873	2888					
2907	2922					
2941	2956					
3009	3024					
3043	3058					
3077	3092					
3094	3109					
3128	3143					
3281	3296					
3315	3330					
3349	3364					
3383	3398					
3417	3432					

3451	3466					
3485	3500					
3519	3534					
3553	3568					
3605	3620					
3639	3654					
3673	3688					
3724	3739					
3758	3773					
3860	3875					
3894	3909					
3962	3977					
4048	4063					
4082	4097					
4133	4148					
4167	4182					
4201	4216					
4320	4335					
4388	4403					
4422	4437					
4456	4471					
2568	2583	CACACTCCCGGGACAC	627	540367	65	eeddddddddddkkk
2636	2651					
2670	2685					
2704	2719					
2738	2753					
2772	2787					
2806	2821					
2840	2855					
2874	2889					
2908	2923					
2942	2957					
3010	3025					
3044	3059					
3078	3093					
3095	3110					
3129	3144					
3282	3297					
3316	3331					
3350	3365					
3384	3399					
3418	3433					
3452	3467					
3486	3501					
3520	3535					
3554	3569					

3606	3621					
3640	3655					
3674	3689					
3725	3740					
3759	3774					
3861	3876					
3895	3910					
3963	3978					
4049	4064					
4083	4098					
4134	4149					
4168	4183					
4202	4217					
4321	4336					
4389	4404					
4423	4438					
4457	4472					
2571	2586	ACCCACACTCCCGGGA	628	540368	55	eeeddddddddddkkk
2639	2654					
2673	2688					
2707	2722					
2741	2756					
2775	2790					
2809	2824					
2843	2858					
2877	2892					
2911	2926					
2945	2960					
3013	3028					
3047	3062					
3081	3096					
3098	3113					
3285	3300					
3319	3334					
3353	3368					
3387	3402					
3421	3436					
3455	3470					
3489	3504					
3523	3538					
3557	3572					
3609	3624					
3643	3658					
3677	3692					
3728	3743					
3762	3777					

3864	3879					
3898	3913					
3966	3981					
4052	4067					
4086	4101					
4137	4152					
4171	4186					
4205	4220					
4324	4339					
4358	4373					
4392	4407					
4426	4441					
4460	4475					
2573	2588	ACACCCACACTCCCGG	629	540369	82	eeeddddddddkkk
2641	2656					
2675	2690					
2709	2724					
2743	2758					
2777	2792					
2811	2826					
2845	2860					
2879	2894					
2913	2928					
2947	2962					
3015	3030					
3049	3064					
3083	3098					
3100	3115					
3287	3302					
3321	3336					
3355	3370					
3389	3404					
3423	3438					
3457	3472					
3491	3506					
3525	3540					
3559	3574					
3611	3626					
3645	3660					
3679	3694					
3730	3745					
3764	3779					
3866	3881					
3900	3915					
3968	3983					
4054	4069					

4088	4103					
4139	4154					
4173	4188					
4207	4222					
4326	4341					
4360	4375					
4394	4409					
4428	4443					
4462	4477					
2576	2591	GGGACACCCACACTCC	630	540370	86	eeeddddddddddkkk
2610	2625					
2644	2659					
2678	2693					
2712	2727					
2746	2761					
2780	2795					
2814	2829					
2848	2863					
2882	2897					
2916	2931					
2950	2965					
3018	3033					
3052	3067					
3086	3101					
3358	3373					
3460	3475					
3562	3577					
3682	3697					
3733	3748					
3869	3884					
3903	3918					
3937	3952					
4091	4106					
4142	4157					
4329	4344					
4431	4446					
4465	4480					
2578	2593	CCGGGACACCCACACT	631	540371	74	eeeddddddddddkkk
2612	2627					
2646	2661					
2680	2695					
2714	2729					
2748	2763					
2782	2797					
2816	2831					
2850	2865					

2884	2899					
2918	2933					
2952	2967					
3020	3035					
3054	3069					
3088	3103					
3360	3375					
3564	3579					
3684	3699					
3735	3750					
3871	3886					
3905	3920					
3939	3954					
4144	4159					
4331	4346					
4433	4448					
4467	4482					
2580	2595	CCCCGGGACACCCACA	632	540372	82	eeeddddddddddkkk
2614	2629					
2648	2663					
2716	2731					
2784	2799					
2818	2833					
2886	2901					
2954	2969					
3022	3037					
3056	3071					
3260	3275					
3362	3377					
3566	3581					
3686	3701					
3703	3718					
3737	3752					
3873	3888					
3941	3956					
4026	4041					
4112	4127					
4146	4161					
4299	4314					
4333	4348					
4435	4450					
4469	4484					
4554	4569					
2581	2596	CCCCCGGGACACCCAC	633	540373	81	eeeddddddddddkkk
2615	2630					
2649	2664					

2717	2732					
2785	2800					
2819	2834					
2887	2902					
2955	2970					
2989	3004					
3023	3038					
3057	3072					
3159	3174					
3176	3191					
3244	3259					
3261	3276					
3363	3378					
3567	3582					
3584	3599					
3687	3702					
3704	3719					
3738	3753					
3840	3855					
3874	3889					
3942	3957					
4027	4042					
4113	4128					
4147	4162					
4249	4264					
4300	4315					
4334	4349					
4436	4451					
4470	4485					
4538	4553					
4555	4570					
2583	2598	CGCCCCCGGGACACCC	634	540374	87	eeeddddddddkkk
2617	2632					
2651	2666					
2787	2802					
2957	2972					
2991	3006					
3025	3040					
3059	3074					
3161	3176					
3178	3193					
3263	3278					
3365	3380					
3569	3584					
3842	3857					
3944	3959					

4115	4130					
4251	4266					
4302	4317					
4438	4453					
4472	4487					
2586	2601	CCACGCCCCCGGGACA	635	540375	78	eeddddddddddkkk
2620	2635					
2654	2669					
2790	2805					
2960	2975					
2994	3009					
3028	3043					
3062	3077					
3147	3162					
3164	3179					
3181	3196					
3266	3281					
3368	3383					
3572	3587					
3845	3860					
3947	3962					
4118	4133					
4254	4269					
4305	4320					
4441	4456					
4475	4490					
2589	2604	CACCCACGCCCCCGGG	636	540376	69	eeddddddddddkkk
2623	2638					
2657	2672					
2793	2808					
2963	2978					
2997	3012					
3031	3046					
3065	3080					
3150	3165					
3167	3182					
3184	3199					
3269	3284					
3371	3386					
3575	3590					
3848	3863					
3950	3965					
4121	4136					
4257	4272					
4308	4323					
4444	4459					

2592	2607	GGACACCCACGCCCC	637	540377	88	eedddddddddkkk
2626	2641					
2660	2675					
2796	2811					
2966	2981					
3000	3015					
3034	3049					
3068	3083					
3153	3168					
3170	3185					
3272	3287					
3374	3389					
3578	3593					
3851	3866					
3953	3968					
4124	4139					
4260	4275					
4311	4326					
4447	4462					
4532	4547					
2593	2608	GGGACACCCACGCCCC	638	540378	85	eedddddddddkkk
2627	2642					
2661	2676					
2797	2812					
2967	2982					
3001	3016					
3035	3050					
3069	3084					
3154	3169					
3171	3186					
3239	3254					
3273	3288					
3375	3390					
3477	3492					
3579	3594					
3852	3867					
3954	3969					
4125	4140					
4261	4276					
4312	4327					
4448	4463					
4533	4548					
2628	2643	CGGGACACCCACGCCC	639	540379	77	eedddddddddkkk
2662	2677					
2798	2813					
2968	2983					

3002	3017					
3036	3051					
3070	3085					
3155	3170					
3172	3187					
3240	3255					
3274	3289					
3376	3391					
3478	3493					
3580	3595					
3853	3868					
3955	3970					
4126	4141					
4262	4277					
4313	4328					
4449	4464					
4534	4549					
2629	2644	CCGGGACACCCACGCC	640	540380	84	eeddddddddddkkk
2663	2678					
2799	2814					
2969	2984					
3003	3018					
3037	3052					
3071	3086					
3156	3171					
3173	3188					
3241	3256					
3275	3290					
3377	3392					
3479	3494					
3581	3596					
3854	3869					
3956	3971					
4127	4142					
4263	4278					
4314	4329					
4450	4465					
4535	4550					
2630	2645	CCCGGGACACCCACGC	641	540381	85	eeddddddddddkkk
2664	2679					
2800	2815					
2970	2985					
2987	3002					
3004	3019					
3038	3053					
3072	3087					

3157	3172					
3174	3189					
3242	3257					
3276	3291					
3378	3393					
3480	3495					
3582	3597					
3838	3853					
3855	3870					
3957	3972					
4128	4143					
4247	4262					
4264	4279					
4315	4330					
4451	4466					
4536	4551					
2683	2698	CCTCCGGGACACCCAC	642	540382	69	eeeddddddddkkk
2751	2766					
2853	2868					
2921	2936					
3806	3821					
3908	3923					
2684	2699	GCCTCCGGGACACCCA	643	540383	85	eeeddddddddkkk
2752	2767					
2854	2869					
2922	2937					
3807	3822					
3909	3924					
2692	2707	ACACCCTCGCCTCCGG	644	540384	88	eeeddddddddkkk
2760	2775					
2862	2877					
2930	2945					
3117	3132					
3338	3353					
3440	3455					
3508	3523					
3542	3557					
3628	3643					
3662	3677					
3781	3796					
3815	3830					
3917	3932					
4190	4205					
4224	4239					
4377	4392					
4411	4426					

2695	2710	GGGACACCCTCGCCTC	645	540385	87	eeedddddddddkkk
2763	2778					
2865	2880					
2933	2948					
3120	3135					
3341	3356					
3443	3458					
3511	3526					
3545	3560					
3631	3646					
3665	3680					
3784	3799					
3818	3833					
3920	3935					
4074	4089					
4193	4208					
4227	4242					
4380	4395					
4414	4429					
2697	2712	CCGGGACACCCTCGCC	646	540386	86	eeedddddddddkkk
2765	2780					
2867	2882					
2935	2950					
3122	3137					
3343	3358					
3445	3460					
3513	3528					
3547	3562					
3633	3648					
3667	3682					
3820	3835					
4076	4091					
4195	4210					
4229	4244					
4382	4397					
4416	4431					
2699	2714	TCCCGGGACACCCTCG	647	540387	77	eeedddddddddkkk
2767	2782					
2869	2884					
2937	2952					
3124	3139					
3345	3360					
3447	3462					
3515	3530					
3549	3564					
3635	3650					

3669	3684					
3822	3837					
4078	4093					
4197	4212					
4231	4246					
4384	4399					
4418	4433					
2701	2716	ACTCCCGGGACACCCT	648	540388	86	eeddddddddddkkk
2769	2784					
2871	2886					
2939	2954					
3126	3141					
3313	3328					
3347	3362					
3415	3430					
3449	3464					
3517	3532					
3551	3566					
3637	3652					
3671	3686					
4080	4095					
4199	4214					
4386	4401					
4420	4435					
2974	2989	CGCTCCCGGGACACCC	649	540389	86	eeddddddddddkkk
3825	3840					
4234	4249					
4268	4283					
4575	4590					
2988	3003	CCCCGGGACACCCACG	650	540390	85	eeddddddddddkkk
3158	3173					
3175	3190					
3243	3258					
3583	3598					
3839	3854					
4248	4263					
4537	4552					
3103	3118	GGAACACCCACACTCC	651	540391	83	eeddddddddddkkk
3290	3305					
3324	3339					
3392	3407					
3426	3441					
3494	3509					
3528	3543					
3614	3629					
3648	3663					

3767	3782					
3971	3986					
4057	4072					
4176	4191					
4210	4225					
4363	4378					
4397	4412					
3106	3121	TCCGGAACACCCACAC	652	540392	43	eeedddddddddkkk
3293	3308					
3327	3342					
3395	3410					
3429	3444					
3497	3512					
3531	3546					
3617	3632					
3651	3666					
3770	3785					
4179	4194					
4213	4228					
4366	4381					
4400	4415					
3109	3124	GCCTCCGGAACACCCA	653	540393	88	eeedddddddddkkk
3194	3209					
3330	3345					
3432	3447					
3500	3515					
3534	3549					
3620	3635					
3654	3669					
3773	3788					
4182	4197					
4216	4231					
4369	4384					
4403	4418					
3112	3127	CTCGCCTCCGGAACAC	654	540394	68	eeedddddddddkkk
3197	3212					
3333	3348					
3435	3450					
3503	3518					
3537	3552					
3623	3638					
3657	3672					
3776	3791					
4185	4200					
4219	4234					
4372	4387					

4406	4421					
3115	3130	ACCCTCGCCTCCGGAA	655	540395	87	eeedddddddddkkk
3200	3215					
3336	3351					
3438	3453					
3506	3521					
3540	3555					
3626	3641					
3660	3675					
3779	3794					
4188	4203					
4222	4237					
4375	4390					
4409	4424	ACCCCCGGGACACCCA	656	540396	87	eeedddddddddkkk
3245	3260					
3585	3600					
3688	3703					
3705	3720					
4028	4043					
4539	4554					
4556	4571	CCACACCCCCGGGACA	657	540397	59	eeedddddddddkkk
3249	3264					
3692	3707					
3709	3724					
4015	4030					
4543	4558	CACCCACACCCCCGGG	658	540398	36	eeedddddddddkkk
3252	3267					
3695	3710					
3712	3727					
4018	4033					
4546	4561	GTGTGTGCATATCTCT	659	540399	81	eeedddddddddkkk
14810	14825					
14886	14901					
14976	14991					

Example 23: High dose tolerability of modified oligonucleotides comprising MOE and cEt modifications targeting human Factor VII in BALB/c mice

BALB/c mice were treated at a high dose with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Additionally, newly designed antisense oligonucleotides were also added to this screen. The newly designed modified antisense oligonucleotides are presented in Table 24 and were designed with the same sequences as antisense oligonucleotides from the study described above. The newly designed

oligonucleotides are 16 nucleosides in length and target intronic repeat regions of SEQ ID NO: 1. The newly designed modified antisense oligonucleotides and their motifs are described in Table 24. The internucleoside linkages throughout each oligonucleotide are phosphorothioate linkages. All cytosines in the oligonucleotides are 5-methylcytosines. The 'Sugar Chemistry' column provides the sugar modifications throughout each oligonucleotide: 'd' indicates a 2'-deoxynucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'e' indicates a 2'-O-methoxyethyl nucleoside. The 'Sequence' column provides the nucleobase sequence for each SEQ ID NO.

Each oligonucleotide listed in Table 24 is targeted to intronic regions of human Factor VII genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_027140.6 truncated from nucleotides 1255000 to 1273000). "Start site" indicates the 5'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the oligonucleotide is targeted in the human gene sequence.

Table 24
Modified antisense oligonucleotides targeted to SEQ ID NO: 1

Start Site on SEQ ID NO: 1	Stop Site on SEQ ID NO: 1	Sequence	SEQ ID NO	ISIS No	Sugar Chemistry
6712	6727	GTGTGAGGTGACCTGT	509	537721	kkkddddddddddeee
6834	6849				
7022	7037				
7140	7155				
7397	7412				
7463	7478				
7862	7877				
6729	6744	TGTGAGGTGTCCTGTG	524	537738	kkkddddddddddeee
6851	6866				
6973	6988				
7039	7054				
7091	7106				
7157	7172				
7209	7224				
7263	7278				
7291	7306				
7350	7365				
7414	7429				
7480	7495				
7512	7527				
7526	7541				
7558	7573				
7630	7645				
7684	7699				
7879	7894				
7911	7926				

7975	7990				
8035	8050				
8067	8082				
8139	8154				
8175	8190				
6762	6777	GGTGACCCGTGAGTGT	539	537759	kkkdddddddddeee
6884	6899				
6950	6965				
7068	7083				
7186	7201				
7240	7255				
6764	6779	GAGGTGACCCGTGAGT	541	537761	kkkdddddddddeee
6886	6901				
6952	6967				
7070	7085				
7188	7203				
7242	7257				
6644	6659	GTGAGGTGACCCGTGA	543	537763	kkkdddddddddeee
6766	6781				
6888	6903				
6954	6969				
7072	7087				
7190	7205				
7244	7259				
6697	6712	TGAGTGTGAAGTGTGC	548	537850	kkkdddddddddeee
6753	6768				
6819	6834				
6875	6890				
6941	6956				
7007	7022				
7059	7074				
7125	7140				
7177	7192				
7382	7397				
7448	7463				
7795	7810				
7945	7960				
8286	8301				
6705	6720	GTGACCTGTGAGTGTG	556	537858	kkkdddddddddeee
6827	6842				
7015	7030				
7133	7148				
7390	7405				
7456	7471				
7606	7621				

7642	7657				
7803	7818				
8115	8130				
8151	8166				
8294	8309				
6711	6726	TGTGAGGTGACCTGTG	562	537864	kkkdddddddddeee
6833	6848				
7021	7036				
7139	7154				
7396	7411				
7462	7477				
7861	7876	TGTGAAGTGTGCCCTG	565	537869	kkkdddddddddeee
6693	6708				
6749	6764				
6815	6830				
6871	6886				
6937	6952				
7055	7070				
7173	7188				
7791	7806	GAGTGTGAAGTGTGCC	568	537872	kkkdddddddddeee
8282	8297				
6696	6711				
6752	6767				
6818	6833				
6874	6889				
6940	6955				
7006	7021				
7058	7073				
7124	7139				
7176	7191				
7381	7396				
7447	7462				
7794	7809				
7944	7959	CTGTGTGAGGTGTCCT	571	537897	kkkdddddddddeee
8285	8300				
6680	6695				
6802	6817				
6924	6939				
7042	7057				
7160	7175				
7515	7530				
7687	7702	TGTGTGAGGTGTCCTG	582	540118	kkkdddddddddeee
8178	8193				
6975	6990				
7041	7056				

7093	7108				
7159	7174				
7352	7367				
7514	7529				
7686	7701				
8177	8192				
7038	7053	GTGAGGTGTCCTGTGG	602	540138	kkkdddddddddeee
7156	7171				
7262	7277				
7479	7494				
7511	7526				
7557	7572				
7910	7925				
7974	7989				
8066	8081	CTGTGAGGTGTCCTGT	603	540139	kkkdddddddddeee
7264	7279				
7415	7430				
7481	7496				
7527	7542				
7880	7895				
7912	7927				
7976	7991	GTGTGAGGTGACCTCT	612	540148	kkkdddddddddeee
7278	7293				
7429	7444				
7495	7510				
7541	7556				
7894	7909				
7926	7941				
7990	8005				
8022	8037	GACCTGTGAGTGTGAG	617	540153	kkkdddddddddeee
7604	7619				
7640	7655				
8113	8128				
8149	8164				
8373	8388	GAGGTGTCCTGTGAGA	619	540155	kkkdddddddddeee
7627	7642				
7663	7678				
7681	7696				
7840	7855				
8136	8151				
8172	8187	ACTCCCGGGACACCCA	624	540162	cekddddddddkke
8331	8346				
2565	2580				
2633	2648				
2667	2682				

2735	2750				
2803	2818				
2837	2852				
2905	2920				
3007	3022				
3041	3056				
3075	3090				
3092	3107				
3279	3294				
3381	3396				
3483	3498				
3603	3618				
3722	3737				
3756	3771				
3858	3873				
3892	3907				
3960	3975				
4046	4061				
4131	4146				
4165	4180				
4318	4333				
4454	4469				
2567	2582	ACACTCCCGGGACACC	626	540164	eekdddddddddkke
2635	2650				
2669	2684				
2703	2718				
2737	2752				
2771	2786				
2805	2820				
2839	2854				
2873	2888				
2907	2922				
2941	2956				
3009	3024				
3043	3058				
3077	3092				
3094	3109				
3128	3143				
3281	3296				
3315	3330				
3349	3364				
3383	3398				
3417	3432				
3451	3466				
3485	3500				

3519	3534				
3553	3568				
3605	3620				
3639	3654				
3673	3688				
3724	3739				
3758	3773				
3860	3875				
3894	3909				
3962	3977				
4048	4063				
4082	4097				
4133	4148				
4167	4182				
4201	4216				
4320	4335				
4388	4403				
4422	4437				
4456	4471				
2576	2591	GGGACACCCACACTCC	630	540168	eekeeeeeeeeeee
2610	2625				
2644	2659				
2678	2693				
2712	2727				
2746	2761				
2780	2795				
2814	2829				
2848	2863				
2882	2897				
2916	2931				
2950	2965				
3018	3033				
3052	3067				
3086	3101				
3358	3373				
3460	3475				
3562	3577				
3682	3697				
3733	3748				
3869	3884				
3903	3918				
3937	3952				
4091	4106				
4142	4157				
4329	4344				

4431	4446				
4465	4480				
2583	2598	CGCCCCCGGGACACCC	634	540172	eekdddddddddkke
2617	2632				
2651	2666				
2787	2802				
2957	2972				
2991	3006				
3025	3040				
3059	3074				
3161	3176				
3178	3193				
3263	3278				
3365	3380				
3569	3584				
3842	3857				
3944	3959				
4115	4130				
4251	4266				
4302	4317				
4438	4453				
4472	4487				
2592	2607	GGACACCCACGCCCC	637	540175	eekdddddddddkke
2626	2641				
2660	2675				
2796	2811				
2966	2981				
3000	3015				
3034	3049				
3068	3083				
3153	3168				
3170	3185				
3272	3287				
3374	3389				
3578	3593				
3851	3866				
3953	3968				
4124	4139				
4260	4275				
4311	4326				
4447	4462				
4532	4547				
2593	2608	GGGACACCCACGCCCC	638	540176	eekdddddddddkke
2627	2642				
2661	2676				

2797	2812				
2967	2982				
3001	3016				
3035	3050				
3069	3084				
3154	3169				
3171	3186				
3239	3254				
3273	3288				
3375	3390				
3477	3492				
3579	3594				
3852	3867				
3954	3969				
4125	4140				
4261	4276				
4312	4327				
4448	4463				
4533	4548				
2629	2644	CCGGGACACCCACGCC	640	540178	eekdddddddddkke
2663	2678				
2799	2814				
2969	2984				
3003	3018				
3037	3052				
3071	3086				
3156	3171				
3173	3188				
3241	3256				
3275	3290				
3377	3392				
3479	3494				
3581	3596				
3854	3869				
3956	3971				
4127	4142				
4263	4278				
4314	4329				
4450	4465				
4535	4550				
2630	2645	CCCGGGACACCCACGC	641	540179	eekdddddddddkke
2664	2679				
2800	2815				
2970	2985				
2987	3002				

3004	3019				
3038	3053				
3072	3087				
3157	3172				
3174	3189				
3242	3257				
3276	3291				
3378	3393				
3480	3495				
3582	3597				
3838	3853				
3855	3870				
3957	3972				
4128	4143				
4247	4262				
4264	4279				
4315	4330				
4451	4466				
4536	4551				
2684	2699	GCCTCCGGGACACCCA	643	540181	eekddddddddkkke
2752	2767				
2854	2869				
2922	2937				
3807	3822				
3909	3924				
2692	2707	ACACCCTCGCCTCCGG	644	540182	eekddddddddkkke
2760	2775				
2862	2877				
2930	2945				
3117	3132				
3338	3353				
3440	3455				
3508	3523				
3542	3557				
3628	3643				
3662	3677				
3781	3796				
3815	3830				
3917	3932				
4190	4205				
4224	4239				
4377	4392				
4411	4426				
2695	2710	GGGACACCCTCGCCTC	645	540183	eekddddddddkkke
2763	2778				

2865	2880				
2933	2948				
3120	3135				
3341	3356				
3443	3458				
3511	3526				
3545	3560				
3631	3646				
3665	3680				
3784	3799				
3818	3833				
3920	3935				
4074	4089				
4193	4208				
4227	4242				
4380	4395				
4414	4429				
2697	2712	CCGGGACACCCTCGCC	646	540184	eekddddddddkkke
2765	2780				
2867	2882				
2935	2950				
3122	3137				
3343	3358				
3445	3460				
3513	3528				
3547	3562				
3633	3648				
3667	3682				
3820	3835				
4076	4091				
4195	4210				
4229	4244				
4382	4397				
4416	4431				
2701	2716	ACTCCCGGGACACCCT	648	540186	eekddddddddkkke
2769	2784				
2871	2886				
2939	2954				
3126	3141				
3313	3328				
3347	3362				
3415	3430				
3449	3464				
3517	3532				
3551	3566				

3637	3652				
3671	3686				
4080	4095				
4199	4214				
4386	4401				
4420	4435				
2974	2989	CGCTCCCGGGACACCC	649	540187	eekdddddddddkke
3825	3840				
4234	4249				
4268	4283				
4575	4590				
2988	3003	CCCCGGGACACCCACG	650	540188	eekdddddddddkke
3158	3173				
3175	3190				
3243	3258				
3583	3598				
3839	3854				
4248	4263				
4537	4552				
3109	3124	GCCTCCGGAACACCCA	653	540191	eekdddddddddkke
3194	3209				
3330	3345				
3432	3447				
3500	3515				
3534	3549				
3620	3635				
3654	3669				
3773	3788				
4182	4197				
4216	4231				
4369	4384				
4403	4418				
3115	3130	ACCCTCGCCTCCGAA	655	540193	eekdddddddddkke
3200	3215				
3336	3351				
3438	3453				
3506	3521				
3540	3555				
3626	3641				
3660	3675				
3779	3794				
4188	4203				
4222	4237				
4375	4390				
4409	4424				

3245	3260	ACCCCCGGGACACCCA	656	540194	eekddddddddddkke
3585	3600				
3688	3703				
3705	3720				
4028	4043				
4539	4554				
4556	4571				
6648	6663	GAGTGTGAGGTGACCC	547	544811	eekddddddddddkke
6770	6785				
6892	6907				
6958	6973				
7076	7091				
7194	7209				
6646	6661	GTGTGAGGTGACCCGT	545	544812	eekddddddddddkke
6768	6783				
6890	6905				
6956	6971				
7074	7089				
7192	7207				
7246	7261				
6732	6747	GAGTGTGAGGTGTCCT	527	544813	eekddddddddddkke
6854	6869				
7212	7227				
7294	7309				
7561	7576				
7633	7648				
8070	8085				
8142	8157				
6706	6721	GGTGACCTGTGAGTGT	557	544814	eekddddddddddkke
6828	6843				
7016	7031				
7134	7149				
7391	7406				
7457	7472				
7607	7622				
7643	7658				
8116	8131				
8152	8167				
6647	6662	AGTGTGAGGTGACCCG	546	544815	eekddddddddddkke
6769	6784				
6891	6906				
6957	6972				
7075	7090				
7193	7208				
6682	6697	CCCTGTGTGAGGTGTC	573	544816	eekddddddddddkke

6804	6819				
6926	6941				
7044	7059				
7162	7177				
7689	7704				
8180	8195				
6681	6696	CCTGTGTGAGGTGTCC	572	544817	eekddddddddddkke
6803	6818				
6925	6940				
7043	7058				
7161	7176				
7516	7531				
7688	7703				
8179	8194				
6694	6709	GTGTGAAGTGTGCCCT	566	544818	eekddddddddddkke
6750	6765				
6816	6831				
6872	6887				
6938	6953				
7056	7071				
7174	7189				
7792	7807				
8283	8298	AGTGTGAGGTGACCTG	510	544819	eekddddddddddkke
6713	6728				
6835	6850				
7398	7413				
7863	7878	GTGTGAGGTGTCCTGT	525	544820	eekddddddddddkke
6730	6745				
6852	6867				
6974	6989				
7040	7055				
7092	7107				
7158	7173				
7210	7225				
7292	7307				
7351	7366				
7513	7528				
7559	7574				
7631	7646				
7685	7700				
8068	8083				
8140	8155				
8176	8191	AGTGTGAAGTGTGCCC	567	544821	eekddddddddddkke
6695	6710				
6751	6766				

6817	6832				
6873	6888				
6939	6954				
7005	7020				
7057	7072				
7123	7138				
7175	7190				
7380	7395				
7446	7461				
7793	7808				
7943	7958				
8284	8299				
6760	6775	TGACCCGTGAGTGTGA	537	544826	eekdddddddddkke
6882	6897				
6948	6963				
7066	7081				
7184	7199				
7238	7253				
7238	7253	GTGACCCGTGAGTGTG	538	544827	eekdddddddddkke
6761	6776				
6883	6898				
6949	6964				
7067	7082				
7185	7200				
7239	7254	GGTGACCCGTGAGTGT	539	544828	eekdddddddddkke
6762	6777				
6884	6899				
6950	6965				
7068	7083				
7186	7201				
7240	7255	AGGTGACCCGTGAGTG	540	544829	eekdddddddddkke
6763	6778				
6885	6900				
6951	6966				
7069	7084				
7187	7202				
7241	7256	GAGGTGACCCGTGAGT	541	544830	eekdddddddddkke
6764	6779				
6886	6901				
6952	6967				
7070	7085				
7188	7203				
7242	7257	TGAGGTGACCCGTGAG	542	545471	eekdddddddddkke
6643	6658				
6765	6780				

6887	6902				
6953	6968				
7071	7086				
7189	7204				
7243	7258				
6644	6659	GTGAGGTGACCCGTGA	543	545472	eekddddddddddkke
6766	6781				
6888	6903				
6954	6969				
7072	7087				
7190	7205				
7244	7259				
6645	6660	TGTGAGGTGACCCGTG	544	545473	eekddddddddddkke
6767	6782				
6889	6904				
6955	6970				
7073	7088				
7191	7206				
7245	7260				
6707	6722	AGGTGACCTGTGAGTG	558	545474	eekddddddddddkke
6829	6844				
7017	7032				
7135	7150				
7392	7407				
7458	7473				
7608	7623				
7644	7659				
8117	8132	GAGGTGACCTGTGAGT	559	545475	eekddddddddddkke
8153	8168				
6708	6723				
6830	6845				
7018	7033				
7136	7151				
7393	7408	TGAGGTGACCTGTGAG	560	545476	eekddddddddddkke
7459	7474				
6709	6724				
6831	6846				
7019	7034				
7137	7152				
7394	7409	GTGAGGTGACCTGTGA	561	545477	eekddddddddddkke
7460	7475				
7859	7874				
6710	6725				
6832	6847				
7020	7035				

7138	7153				
7395	7410				
7461	7476				
7860	7875				
6711	6726	TGTGAGGTGACCTGTG	562	545478	eekddddddddkkke
6833	6848				
7021	7036				
7139	7154				
7396	7411				
7462	7477				
7861	7876				
6705	6720	GTGACCTGTGAGTGTG	556	545479	eekddddddddkkke
6827	6842				
7015	7030				
7133	7148				
7390	7405				
7456	7471				
7606	7621				
7642	7657				
7803	7818				
8115	8130				
8151	8166				
8294	8309				
6718	6733	CTGTGAGTGTGAGGTG	514	537727	kkkdddddddddeee
6736	6751				
6840	6855				
6858	6873				
6962	6977				
7080	7095				
7198	7213				
7339	7354				
7403	7418				
7637	7652				
7868	7883				
8146	8161				
8393	8408				

Treatment

Male BALB/c mice were injected subcutaneously with a single dose of 200 mg/kg of ISIS 422142, ISIS 457851, ISIS 473294, ISIS 473295, ISIS 473327, ISIS 484714, ISIS 515334, ISIS 515338, ISIS 515354, ISIS 515366, ISIS 515380, ISIS 515381, ISIS 515382, ISIS 515384, ISIS 515386, ISIS 515387, ISIS 515388, ISIS 515406, ISIS 515407, ISIS 515408, ISIS 515422, ISIS 515423, ISIS 515424, ISIS 515532, ISIS 515534,

ISIS 515538, ISIS 515539, ISIS 515558, ISIS 515656, ISIS 515575, ISIS 515926, ISIS 515944, ISIS 515945,
 ISIS 515948, ISIS 515949, ISIS 515951, ISIS 515952, ISSI 516003, ISIS 516055, ISIS 516057, ISIS 516060,
 ISIS 516062, ISIS 529126, ISIS 529146, ISIS 529166, ISIS 529170, ISIS 529172, ISIS 529173, ISIS 529174,
 ISIS 529175, ISSI 529176, ISIS 529182, ISIS 529183, ISIS 529186, ISIS 529282, ISIS 529304, ISIS 529306,
 5 ISIS 529360, ISIS 529450, ISIS 529459, ISIS 529460, ISIS 529461, ISIS 529547, ISIS 529550, ISIS 529551,
 ISIS 529553, ISIS 529557, ISIS 529562, ISIS 529563, ISIS 529564, ISIS 529565, ISIS 529575, ISIS 529582,
 ISIS 529589, ISIS 529607, ISIS 529614, ISIS 529632, ISIS 529650, ISIS 529651, ISIS 529657, ISIS 529663,
 ISIS 529725, ISIS 529745, ISIS 529765, ISIS 529785, ISIS 529804, ISIS 529818, ISIS 529823, ISIS 529854,
 ISIS 534528, ISIS 534534, ISIS 534594, ISIS 534660, ISIS 534663, ISIS 534664, ISIS 534676, ISIS 534677,
 10 ISIS 537679, ISIS 537683, ISIS 534693, ISIS 534701, ISIS 534716, ISIS 534730, ISIS 534765, ISIS 534795,
 ISIS 534796, ISIS 534797, ISIS 534798, ISIS 534799, ISIS 534800, ISIS 534802, ISIS 534806, ISSI 534830,
 ISIS 534838, ISIS 534888, ISIS 534890, ISIS 534898, ISIS 534911, ISIS 534920, ISIS 534926, ISIS 534937,
 ISIS 534950, ISSI 534956, ISIS 534980, ISIS 534986, ISIS 535010, ISIS 535043, ISIS 535049, ISIS 535076,
 ISIS 535082, ISSI 535142, ISIS 537024, ISIS 537030, ISIS 537041, ISIS 537062, ISIS 537064, ISIS 537066,
 15 ISIS 537721, ISIS 537727, ISIS 537738, ISIS 537759, ISIS 537761, ISIS 537763, ISIS 537792, ISIS 537800,
 ISIS 537806, ISIS 537811, ISIS 537814, ISIS 537839, ISIS 537850, ISSI 537858, ISIS 537864, ISIS 537869,
 ISIS 537872, ISIS 537897, ISIS 538160, ISIS 538196, ISIS 538205, ISIS 538228, ISIS 538242, ISIS 538361,
 ISIS 538380, ISIS 540118, ISIS 540138, ISIS 540139, ISIS 540148, ISIS 540153, ISIS 540155, ISIS 540162,
 ISIS 540164, ISIS 540168, ISIS 540172, ISIS 540175, ISIS 540176, ISIS 540178, ISIS 540179, ISIS 540181,
 20 ISIS 540182, ISIS 540183, ISIS 540184, ISIS 540186, ISIS 540187, ISIS 540188, ISIS 540191, ISIS 540193,
 ISIS 540194, ISIS 544811, ISIS 544812, ISIS 544813, ISIS 544814, ISIS 544815, ISIS 544816, ISIS 544817,
 ISIS 544818, ISIS 544819, ISIS 544820, ISIS 544821, ISIS 544826, ISIS 544827, ISIS 544828, ISIS 544829,
 ISIS 544830, ISIS 545471, ISIS 545472, ISIS 545473, ISIS 545474, ISIS 545475, ISIS 545476, ISIS 545477,
 ISIS 545478, or ISIS 545479. One set of male BALB/c mice was injected with a single dose of PBS. Mice
 25 were euthanized 96 hours later, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of
 transaminases, bilirubin, albumin, and BUN were measured using an automated clinical chemistry analyzer
 (Hitachi Olympus AU400e, Melville, NY).

30 ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused
 an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS
 oligonucleotides that caused an increase in the levels of transaminases between three times and seven times
 the ULN were deemed tolerable. Based on these criteria, ISIS 529166, ISIS 529170, ISIS 529175, ISIS
 529176, ISIS 529186, ISIS 529282, ISIS 529360, ISIS 529450, ISIS 529459, ISIS 529460, ISIS 529547, ISIS
 35 529549, ISIS 529551, ISIS 529553, ISIS 529557, ISIS 529562, ISIS 529575, ISIS 529582, ISIS 529607, ISIS

529589, ISIS 529632, ISIS 529657, ISIS 529725, ISIS 529745, ISIS 529785, ISIS 529799, ISIS 529804, ISIS 529818, ISIS 529823, ISIS 534950, ISIS 534980, ISIS 535010, ISIS 537030, ISIS 537041, ISIS 537062, ISIS 537064, ISIS 537066, ISIS 537759, ISIS 537792, ISIS 537800, ISIS 537839, ISIS 538228, ISIS 473294, ISIS 473295, ISIS 484714, ISIS 515338, ISIS 515366, ISIS 515380, ISIS 515381, ISIS 515387, ISIS 515408, ISIS 515423, ISIS 515424, ISIS 515532, ISIS 515534, ISIS 515538, ISIS 515539, ISIS 515558, ISIS 515575, ISIS 515926, ISIS 515944, ISIS 515945, ISIS 515951, ISIS 515952, ISIS 529126, ISIS 529765, ISIS 534528, ISIS 534534, ISIS 534594, ISIS 534663, ISIS 534676, ISIS 534677, ISIS 534679, ISIS 534683, ISIS 534693, ISIS 534701, ISIS 534716, ISIS 534730, ISIS 534806, ISIS 534830, ISIS 534838, ISIS 534890, ISIS 534898, ISIS 534911, ISIS 534937, ISIS 534956, ISIS 534986, ISIS 535043, ISIS 535049, ISIS 535076, ISIS 535082, ISIS 535142, ISIS 538160, ISIS 538242, ISIS 538361, ISIS 538380, ISIS 534795, ISIS 534796, ISIS 534797, ISIS 540162, ISIS 540164, ISIS 540168, ISIS 540172, ISIS 540175, ISIS 540176, ISIS 540178, ISIS 540179, ISIS 540181, ISIS 540182, ISIS 540183, ISIS 540184, ISIS 540186, ISIS 540187, ISIS 540188, ISIS 540191, ISIS 540193, ISIS 540194, ISIS 544813, ISIS 544814, ISIS 544816, ISIS 544826, ISIS 544827, ISIS 544828, ISIS 544829, ISIS 545473, and ISIS 545474 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 529173, ISIS 529854, ISIS 529614, ISIS 515386, ISIS 515388, ISIS 515949, ISIS 544817, and ISIS 545479 were considered tolerable in terms of liver function.

Example 24: Tolerability of modified antisense oligonucleotides targeting human Factor VII in Sprague-Dawley rats

Sprague-Dawley rats are a multipurpose model used for safety and efficacy evaluations. The rats were treated with ISIS antisense oligonucleotides from the studies described in the Examples above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Six to eight week old male Sprague-Dawley rats were maintained on a 12-hour light/dark cycle and fed ad libitum with Teklad normal rat chow. Groups of four Sprague-Dawley rats each were injected subcutaneously twice a week for 6 weeks with 25 mg/kg of ISIS 473286, ISIS 473547, ISIS 473567, ISIS 473589, ISIS 473630, ISIS 484559, ISIS 515636, ISIS 515640, ISIS 515641, ISIS 515655, ISIS 515657, ISIS 516046, ISIS 516048, ISIS 516051, ISIS 516052, or ISIS 516062. A group of four Sprague-Dawley rats was injected subcutaneously twice a week for 6 weeks with PBS. Forty-eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured. Plasma levels of Bilirubin and BUN were also measured using the same clinical chemistry analyzer.

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS oligonucleotides that caused an increase in the levels of transaminases between three times and seven times the ULN were deemed tolerable. Based on these criteria, ISIS 473286, ISIS 473547, ISSI 473589, ISSI 473630, ISIS 484559, ISIS 515636, ISIS 515640, ISIS 515655, ISIS 516046, and ISIS 516051 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 473567, ISIS 515641, ISIS 515657, ISIS 516048, and ISIS 516051 were considered tolerable in terms of liver function.

Example 25: Tolerability of modified antisense oligonucleotides comprising MOE modifications targeting human Factor VII in Sprague-Dawley rats

Sprague-Dawley rats were treated with ISIS antisense oligonucleotides from the studies described in the Examples above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Six-eight week old male Sprague-Dawley rats were maintained on a 12-hour light/dark cycle and fed ad libitum with Purina normal rat chow. Groups of four Sprague-Dawley rats each were injected subcutaneously twice a week for 6 weeks with 50 mg/kg of ISIS 407936, ISIS 416507, ISIS 416508, ISIS 490208, ISIS 490279, ISIS 490323, ISIS 490368, ISIS 490396, ISIS 490803, ISIS 491122, ISIS 513419, ISIS 513446, ISIS 513454, ISIS 513455, ISIS 513456, ISIS 513504, ISIS 513507, or ISIS 513508. A group of four Sprague-Dawley rats was injected subcutaneously twice a week for 6 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of Bilirubin and BUN were also measured using the same clinical chemistry analyzer.

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS oligonucleotides that caused an increase in the levels of transaminases between three times and seven times the ULN were deemed tolerable. Based on these criteria, ISIS 416507, ISIS 490208, ISIS 490368, ISIS 490396, ISIS 490803, ISIS 491122, ISIS 513446, ISIS 513454, ISIS 513455, ISIS 513456, ISIS 513504, and ISIS 513508 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 407936, ISIS 416508, ISIS 490279, and ISIS 513507 were considered tolerable in terms of liver function.

Example 26: Tolerability of modified oligonucleotides comprising MOE modifications targeting human Factor VII in CD-1 mice

CD-1 mice are a multipurpose mice model, frequently utilized for safety and efficacy testing. The mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

5 Groups of 3 male CD-1 mice each were injected subcutaneously twice a week for 6 weeks with 50 mg/kg of ISIS 473244, ISIS 473295, ISIS 484714, ISIS 515386, ISIS 515424, ISIS 515534, ISIS 515558, ISIS 515926, ISIS 515949, ISIS 515951, ISIS 515952, ISIS 529126, ISIS 529166, ISIS 529173, ISIS 529186, ISIS 529360, ISIS 529461, ISIS 529553, ISIS 529564, ISIS 529582, ISIS 529614, ISIS 529725, ISIS 529745, ISIS 529765, ISIS 529785, ISIS 529799, ISIS 529818, ISIS 529823, ISIS 534528, ISIS 534594, or ISIS
10 534664. One group of male CD-1 mice was injected subcutaneously twice a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, bilirubin, albumin, and BUN were measured using an automated clinical chemistry analyzer
15 (Hitachi Olympus AU400e, Melville, NY).

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS oligonucleotides that caused an increase in the levels of transaminases between three times and seven times the ULN were deemed tolerable. Based on these criteria, ISIS 473295, ISIS 473714, ISIS 515558, ISIS
20 515926, 515951, ISIS 515952, ISIS 529126, ISIS 529166, 529564, ISIS 529582, ISIS 529614, ISIS 529725, ISIS 529765, ISIS 529799, ISIS 529823, and ISIS 534594 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 515424, ISIS 515534, ISIS 515926, ISIS 529785, and ISIS 534664 were considered tolerable in terms of liver function.

Example 27: Tolerability of modified oligonucleotides comprising MOE modifications targeting human 25 Factor VII in CD-1 mice

CD-1 mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Groups of 3 male CD-1 mice each were injected subcutaneously twice a week for 6 weeks with 100
30 mg/kg of ISIS 490208, ISIS 490279, ISIS 490323, ISIS 490368, ISIS 490396, ISIS 490803, ISIS 491122, ISIS 513419, ISIS 513446, ISIS 513454, ISIS 513455, ISIS 513456, ISIS 513504, ISIS 513507, or ISIS 513508. Groups of 3 male CD-1 mice each were injected subcutaneously twice a week for 6 weeks with 100 mg/kg of ISIS 407936, ISIS 416507, or ISIS 416508, which are gapmers described in a previous publication.

One group of male CD-1 mice was injected subcutaneously twice a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, bilirubin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS oligonucleotides that caused an increase in the levels of transaminases between three times and seven times the ULN were deemed tolerable. Based on these criteria, ISIS 407936, ISIS 416507, ISIS 490279, ISIS 490368, ISIS 490396, ISIS 490803, ISIS 491122, ISIS 513446, ISIS 513454, ISIS 513456, and ISIS 513504 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 490208, ISIS 513455, ISIS 513507, and ISIS 513508 were considered tolerable in terms of liver function.

Example 28: Efficacy of modified oligonucleotides comprising MOE and cEt modifications targeting human Factor VII in transgenic mice

Transgenic mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for efficacy.

Treatment

Groups of 2-3 male and female transgenic mice were injected subcutaneously twice a week for 3 weeks with 2.5 mg/kg of ISIS 473244, ISIS 473295, ISIS 484714, ISIS 515926, ISIS 515951, ISIS 515952, ISIS 516062, ISIS 529126, ISIS 529553, ISIS 529745, ISIS 529799, ISIS 534664, ISIS 534826, ISIS 540168, ISIS 540175, ISIS 544826, ISIS 544827, ISIS 544828, or ISIS 544829. One group of mice was injected subcutaneously twice a week for 3 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Protein Analysis

Plasma protein levels of Factor VII were estimated using a Zymutest FVII ELISA kit (Hyphen Bio-Med cat# ARK036A). Results are presented as percent inhibition of Factor VII, relative to control. As shown in Table 25, several antisense oligonucleotides achieved significant reduction of human Factor VII over the PBS control. 'n.d.' indicates that the value for that particular oligonucleotide was not measured.

Table 25
Percent inhibition of Factor VII plasma protein levels in transgenic mice

ISIS No	% inhibition
473244	2
473295	13
484714	19
515926	11
515951	13
515952	0
516062	62
529126	0
529553	0
529745	22
529799	26
534664	32
534826	n.d.
540168	94
540175	98
544813	0
544826	23
544827	60
544828	33
544829	53

Example 29: Efficacy of modified oligonucleotides comprising MOE and cEt modifications targeting human Factor VII in transgenic mice

Transgenic mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for efficacy.

Treatment

Groups of 2-3 male and female transgenic mice were injected subcutaneously twice a week for 3 weeks with 0.5 mg/kg of ISIS 407936, ISIS 490197, ISIS 490275, ISIS 490278, ISIS 490279, ISIS 490323, ISIS 490368, ISIS 490396, ISIS 490803, ISIS 491122, ISIS 513446, ISIS 513447, ISIS 513504, ISIS 516062, ISIS 529166, ISIS 529173, ISIS 529360, ISIS 529725, ISIS 534557, ISIS 534594, ISIS 534664, ISIS 534688, ISIS 534689, ISIS 534915, ISIS 534916, ISIS 534917, or ISIS 534980. One group of mice was injected

subcutaneously twice a week for 3 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Protein Analysis

5 Plasma protein levels of Factor VII were estimated using a Zymutest FVII ELISA kit (Hyphen Bio-Med cat# ARK036A). Results are presented as percent inhibition of Factor VII, relative to control. As shown in Table 26, several antisense oligonucleotides achieved significant reduction of human Factor VII over the PBS control.

Table 26

Percent inhibition of Factor VII plasm protein levels in transgenic mice

ISIS No	% inhibition
407936	28
490197	50
490275	21
490278	20
490279	59
490323	54
490368	22
490396	31
490803	30
491122	51
513446	29
513447	44
513504	45
516062	75
529166	37
529173	64
529360	43
529725	53
534557	76
534594	40
534664	14
534687	12
534688	48

534689	25
534915	40
534916	45
534917	66
534980	62

Example 30: Tolerability of antisense oligonucleotides targeting human Factor VII in Sprague-Dawley rats

Sprague-Dawley rats were treated with ISIS antisense oligonucleotides from the studies described in the Examples above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Six to eight week old male Sprague-Dawley rats were maintained on a 12-hour light/dark cycle and fed ad libitum with Teklad normal rat chow. Groups of four Sprague-Dawley rats each were injected subcutaneously twice a week for 4 weeks with ISIS 515380, ISIS 515381, ISIS 515387, ISIS 529175, ISIS 529176, ISIS 529575, ISIS 529804, or ISIS 537064. Doses 1, 5, 6, 7, and 8 were 25 mg/kg; dose 2 was 75 mg/kg; doses 3 and 4 were 50 mg/kg. One group of four Sprague-Dawley rats was injected subcutaneously twice a week for 4 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured. Plasma levels of Bilirubin and BUN were also measured using the same clinical chemistry analyzer.

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused increase in the levels within three times the upper limit of normal levels of transaminases were deemed very tolerable. ISIS oligonucleotides that caused increase in the levels of transaminases between three times and seven times the upper limit of normal levels were deemed tolerable. Based on these criteria, ISIS 515380, ISIS 515387, ISIS 529175, ISIS 529176, ISIS 529804, and ISIS 537064 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 515381 was considered tolerable in terms of liver function.

Example 31: Efficacy of modified antisense oligonucleotides targeting human Factor VII in transgenic mice

Transgenic mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for efficacy.

Treatment

Two groups of 3 male and female transgenic mice were injected subcutaneously twice a week for 2 weeks with 0.25 mg/kg or 0.75 mg/kg of ISIS 407935 or ISIS 513455. Another group of mice was subcutaneously twice a week for 2 weeks with 0. mg/kg or 1.0 mg/kg of ISIS 473286. Another 16 groups of mice were subcutaneously twice a week for 2 weeks with 0.05 mg/kg or 0.15 mg/kg of ISIS 473589, ISIS 515380, ISIS 515423, ISIS 529804, ISIS 534676, ISIS 534796, ISIS 540162, ISIS 540164, ISIS 540175, ISIS 540179, ISIS 540181, ISIS 540182, ISIS 540186, ISIS 540191, ISIS 540193, ISIS 544827, or ISIS 545474. Another 3 groups of mice were injected subcutaneously twice a week for 2 weeks with 0.15 mg/kg of ISIS 516062, ISIS 534528 or ISIS 534693. One group of mice was injected subcutaneously twice a week for 2 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Protein Analysis

Plasma protein levels of Factor VII were estimated using a Zymutest FVII ELISA kit (Hyphen Bio-Med cat# ARK036A). Results are presented as percent inhibition of Factor VII, relative to control. As shown in Table 27, several antisense oligonucleotides achieved significant reduction of human Factor VII over the PBS control.

Table 27

Percent inhibition of Factor VII plasma protein levels in transgenic mice

ISIS No	Dose (mg/kg/wk)	% inhibition
407935	1.5	65
	0.5	31
513455	1.5	64
	0.5	52
473286	2.0	67
	0.6	11
473589	0.3	42
	0.1	12
515380	0.3	64
	0.1	32
515423	0.3	72
	0.1	37
529804	0.3	36

	0.1	24
534676	0.3	31
	0.1	18
534796	0.3	54
	0.1	43
540162	0.3	84
	0.1	42
540164	0.3	25
	0.1	17
540175	0.3	90
	0.1	55
540179	0.3	29
	0.1	24
540181	0.3	53
	0.1	0
540182	0.3	78
	0.1	21
540186	0.3	72
	0.1	46
540191	0.3	62
	0.1	35
540193	0.3	74
	0.1	46
544827	0.3	28
	0.1	19
545474	0.3	59
	0.1	0
516062	0.3	33
534528	0.3	41
534693	0.3	34

Example 32: Tolerability of antisense oligonucleotides targeting human Factor VII in Sprague-Dawley rats

Sprague-Dawley rats were treated with ISIS antisense oligonucleotides from the studies described in the Examples above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Five-six week old male Sprague-Dawley rats were maintained on a 12-hour light/dark cycle and fed ad libitum with Teklad normal rat chow. Groups of four Sprague-Dawley rats each were injected subcutaneously twice a week for 4 weeks with 50 mg/kg of ISIS 515423, ISIS 515424, ISIS 515640, ISIS 534676, ISIS 534796, ISIS 534797, ISIS 540162, ISIS 540164, ISIS 540172, ISIS 540175, ISIS 540179, ISIS 540181, ISIS 540182, ISIS 540183, ISIS 540186, ISIS 540191, or ISIS 545474. A group of four Sprague-Dawley rats was injected subcutaneously twice a week for 4 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured. Plasma levels of Bilirubin and BUN were also measured using the same clinical chemistry analyzer.

ISIS oligonucleotides that did not cause any increase in the levels of transaminases, or which caused an increase within three times the upper limit of normal (ULN) were deemed very tolerable. ISIS oligonucleotides that caused an increase in the levels of transaminases between three times and seven times the ULN were deemed tolerable. Based on these criteria, ISIS 540164, ISIS 540172, and ISIS 540175 were considered very tolerable in terms of liver function. Based on these criteria, ISIS 534676, ISIS 534796, ISIS 534797, ISIS 540162, and ISIS 540179 were considered tolerable in terms of liver function.

Example 33: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Antisense oligonucleotides selected from the studies described above were tested at various doses in Hep3B cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.05 μ M, 0.15 μ M, 0.44 μ M, 1.33 μ M, and 4.00 μ M concentrations of antisense oligonucleotide, as specified in Table 28. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Human Factor VII primer probe set RTS2927 was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 28. As illustrated in Table 28, Factor VII mRNA levels were significantly reduced in a dose-dependent manner in several of the antisense oligonucleotide treated cells.

Table 28
Dose-dependent antisense inhibition (%) of human Factor VII in Hep3B cells using electroporation

ISIS No	0.05 μ M	0.15 μ M	0.44 μ M	1.33 μ M	4.00 μ M	IC ₅₀ (μ M)
473286	0	1	13	12	15	>4.0

457851	23	32	57	80	93	0.3
473286	3	20	43	71	88	0.5
473286	15	26	24	28	36	>4.0
473286	6	3	10	26	29	>4.0
473327	14	28	35	67	90	0.5
473589	29	53	76	89	95	0.1
515380	44	72	85	93	95	<0.05
515423	43	64	87	95	98	<0.05
515424	38	55	85	92	97	0.1
515636	21	33	74	82	93	0.2
516046	29	23	29	48	78	0.9
516048	35	24	41	67	87	0.4
516052	18	6	48	63	80	0.6
516062	24	14	21	47	68	1.6
529166	16	47	75	87	94	0.2
529173	14	49	77	91	96	0.2
529175	30	69	88	93	96	0.1
529176	34	63	85	93	96	0.1
529360	35	53	74	91	93	0.1
529725	53	69	85	92	95	<0.05
529804	37	41	71	90	94	0.1
534528	50	68	78	93	97	<0.05
534557	48	78	90	94	95	<0.05
534594	39	47	76	87	94	0.1
534676	29	20	40	64	87	0.5
534687	41	37	56	80	93	0.2
534688	16	56	88	94	96	0.1
534689	21	59	82	94	95	0.1
534693	18	58	81	93	95	0.1
534795	19	43	68	90	94	0.2
534796	25	59	80	93	96	0.1
534890	31	55	77	90	96	0.1
534898	22	61	80	94	97	0.1
534915	19	26	51	77	94	0.3
534916	20	36	66	86	93	0.2
534917	34	53	82	89	94	0.1
540162	40	64	84	90	92	<0.05
540164	34	60	83	91	92	0.1
540168	51	79	90	92	94	<0.05
540172	40	66	80	88	92	<0.05
540175	30	61	80	88	91	0.1
540176	7	17	50	75	85	0.5
540179	11	22	25	16	19	>4.0

540181	19	46	72	86	91	0.2
540182	16	66	83	86	92	0.1
540183	39	74	87	92	93	<0.05
540186	31	69	85	91	94	0.1
540191	38	54	80	88	91	0.1
540193	57	67	84	94	97	<0.05
540194	30	45	62	77	91	0.2
544827	37	42	67	82	96	0.1
544829	26	41	42	71	93	0.3
545473	28	27	49	80	97	0.3
545474	23	27	55	84	96	0.3

Example 34: Tolerability of antisense oligonucleotides targeting human Factor VII in CD-1 mice

CD-1 mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Two groups of 4 male 6-8 week old CD-1 mice each were injected subcutaneously twice a week for 6 weeks with 50 mg/kg of ISIS 407935 or ISIS 490279. Another seven groups of 4 male 6-8 week old CD-1 mice each were injected subcutaneously twice a week for 6 weeks with 25 mg/kg of ISIS 473589, ISIS 529804, ISIS 534796, ISIS 540162, ISIS 540175, ISIS 540182, or ISIS 540191. One group of male CD-1 mice was injected subcutaneously twice a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, bilirubin, albumin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in Table 29. 'MOE' indicates that the antisense oligonucleotide is a MOE gapmer. 'DMC' indicates that the antisense oligonucleotide comprises deoxy, cEt, and MOE modifications. Treatment with the newly designed antisense oligonucleotides are more tolerable compared to treatment with ISIS 407935 (disclosed in an earlier publication), which caused elevation of ALT levels greater than seven times the upper limit of normal (ULN).

Table 29

Effect of antisense oligonucleotide treatment on liver function in CD-1 mice

	Chemistry	Dose (mg/kg/wk)	ALT(IU/L)	AST (IU/L)	BUN (mg/dL)	Bilirubin (mg/dL)
PBS	-	-	37	47	28	0.2
407935	MOE	100	373	217	24	0.2
490279	MOE	100	96	82	24	0.2
473589	DMC	50	93	116	22	0.2

529804	DMC	50	54	74	27	0.2
534796	DMC	50	60	63	27	0.2
540162	DMC	50	43	55	29	0.2
540175	DMC	50	113	78	24	0.3
540182	DMC	50	147	95	26	0.1
540191	DMC	50	79	88	28	0.2

Body and organ weights

Body weights, as well as liver, heart, lungs, spleen and kidney weights were measured at the end of the study, and are presented in Table 30. MOE' indicates that the antisense oligonucleotide is a MOE gapmer. 'DMC' indicates that the antisense oligonucleotide comprises deoxy, cEt and MOE modifications. Several of the ISIS oligonucleotides did not cause any changes in organ weights outside the expected range and were therefore deemed tolerable in terms of organ weights.

Table 30
Body and organ weights (grams) of CD-1 mice

	Chemistry	Dose (mg/kg/wk)	Body weight	Liver	Spleen	Kidney
PBS	-	-	42	2.2	0.12	0.64
407935	MOE	100	40	2.6	0.20	0.62
490279	MOE	100	42	2.8	0.17	0.61
473589	DMC	50	41	2.5	0.16	0.67
529804	DMC	50	40	2.3	0.14	0.62
534796	DMC	50	37	2.6	0.15	0.51
540162	DMC	50	42	2.4	0.15	0.60
540175	DMC	50	39	2.2	0.11	0.62
540182	DMC	50	41	2.6	0.16	0.61
540191	DMC	50	40	2.4	0.13	0.60

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

Sprague-Dawley rats were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Two groups of 4 male 7-8 week old Sprague-Dawley rats each were injected subcutaneously twice a week for 6 weeks with 50 mg/kg of ISIS 407935 or ISIS 490279. Another seven groups of 4 male 6-8 week old Sprague-Dawley rats each were injected subcutaneously twice a week for 6 weeks with 25 mg/kg of ISIS 473589, ISIS 529804, ISIS 534796, ISIS 540162, ISIS 540175, ISIS 540182, or ISIS 540191. One group of

male Sprague-Dawley rats was injected subcutaneously twice a week for 6 weeks with PBS. The rats were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, bilirubin, albumin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in Table 31. MOE' indicates that the antisense oligonucleotide is a MOE gapmer. 'DMC' indicates that the antisense oligonucleotide comprises deoxy, cEt and MOE modifications. Treatment with the all antisense oligonucleotides was tolerable in terms of plasma chemistry markers in this model.

Table 31

Effect of antisense oligonucleotide treatment on liver function in Sprague-Dawley rats

	Chemistry	Dose (mg/kg/wk)	ALT(IU/L)	AST (IU/L)	BUN (mg/dL)	Bilirubin (mg/dL)
PBS	-	-	71	83	19	0.2
407935	MOE	100	74	96	22	0.2
490279	MOE	100	96	181	22	0.4
473589	DMC	50	57	73	21	0.2
529804	DMC	50	54	78	21	0.2
534796	DMC	50	68	98	22	0.2
540162	DMC	50	96	82	21	0.1
540175	DMC	50	55	73	18	0.2
540182	DMC	50	45	87	21	0.2
540191	DMC	50	77	104	21	0.2

Body and organ weights

Body weights, as well as liver, heart, lungs, spleen and kidney weights were measured at the end of the study, and are presented in Table 32. MOE' indicates that the antisense oligonucleotide is a MOE gapmer. 'DMC' indicates that the antisense oligonucleotide comprises deoxy, cEt and MOE modifications. Treatment with all the antisense oligonucleotides was tolerable in terms of body and organ weights in this model.

Table 32

Body and organ weights (grams) of Sprague-Dawley rats

	Chemistry	Dose (mg/kg/wk)	Body weight	Liver	Spleen	Kidney
PBS		-	443	16	0.8	3.5
ISIS 407935	MOE	100	337	14	1.8	3.2
ISIS 490279	MOE	100	365	18	2.2	2.9
ISIS 473589	DMC	50	432	18	1.3	3.3
ISIS 529804	DMC	50	429	18	2.2	3.4

ISIS 534796	DMC	50	434	15	1.4	3.3
ISIS 540162	DMC	50	446	18	1.1	3.3
ISIS 540175	DMC	50	467	16	1.0	3.5
ISIS 540182	DMC	50	447	22	2.5	4.5
ISIS 540191	DMC	50	471	21	1.4	3.9

Example 36: Dose-dependent antisense inhibition of human Factor VII in cynomolgus monkey primary hepatocytes

Antisense oligonucleotides selected from the studies described above were tested at various doses in cynomolgous monkey primary hepatocytes. Cells were plated at a density of 35,000 cells per well and transfected using electroporation with 0.009 μ M, 0.03 μ M, 0.08 μ M, 0.25 μ M, 0.74 μ M, 2.22 μ M, 6.67 μ M, and 20.00 μ M concentrations of antisense oligonucleotide, as specified in Table 33. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Factor VII primer probe set RTS2927 was used to measure mRNA levels.

Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. As illustrated in Table 33, Factor VII mRNA levels were significantly reduced in a dose-dependent manner with some of the antisense oligonucleotides that are cross-reactive with the rhesus monkey genomic sequence (GENBANK Accession No. NW_001104507.1 truncated from nucleotides 691000 to 706000; SEQ ID NO: 4). 'n/a' indicates that the antisense oligonucleotide has more than 3 mismatches with SEQ ID NO: 4.

Table 33

Dose-dependent antisense inhibition (%) of Factor VII in cynomolgous monkey primary hepatocytes using electroporation

ISIS No	Start Site on SEQ ID NO: 4	0.009 μ M	0.03 μ M	0.08 μ M	0.25 μ M	0.74 μ M	2.22 μ M	6.67 μ M	20.00 μ M
490279	808	19	12	13	0	6	18	27	22
473589	12845	5	10	19	42	64	76	88	92
529804	12909	10	3	23	25	57	80	86	91
534796	12848	0	28	23	49	71	81	87	90
540162	2358	9	14	9	6	13	13	11	31
540175	2051	0	4	12	9	10	16	12	22
	2285								
540182	n/a	0	7	0	6	36	12	10	0
540191	n/a	6	7	0	0	0	0	21	42
407935	n/a	10	18	15	29	56	73	82	88

Example 37: Dose-dependent antisense inhibition of human Factor VII in Hep3B cells

Antisense oligonucleotides from the study described above were also tested at various doses in Hep3B cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.009 μ M, 0.03 μ M, 0.08 μ M, 0.25 μ M, 0.74 μ M, 2.22 μ M, 6.67 μ M, and 20.00 μ M concentrations of antisense oligonucleotide, as specified in Table 34. After a treatment period of approximately 16 hours, RNA was isolated from the cells and Factor VII mRNA levels were measured by quantitative real-time PCR. Factor VII primer probe set RTS2927 was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of Factor VII expression, relative to untreated control cells. As illustrated in Table 34, Factor VII mRNA levels were significantly reduced in a dose-dependent manner with several of the antisense oligonucleotides.

Table 34

Dose-dependent antisense inhibition (%) of Factor VII in Hep3B cells using electroporation

ISIS No	0.009 μ M	0.03 μ M	0.08 μ M	0.25 μ M	0.74 μ M	2.22 μ M	6.67 μ M	20.00 μ M	IC ₅₀ (μ M)
407935	3	9	11	35	64	83	87	93	4.5
473244	20	33	50	69	77	89	7	14	0.9
473589	0	14	23	44	74	88	90	94	2.7
490279	0	5	7	15	25	61	76	78	11.6
515952	0	12	27	57	76	89	93	94	2.2
516066	6	0	12	26	52	70	81	86	6.0
529459	0	4	24	40	61	78	88	94	3.5
529553	9	7	17	40	58	74	87	93	4.6
529804	0	3	34	64	83	89	93	95	2.0
534796	8	18	43	67	82	89	95	96	1.4
537806	6	11	5	20	37	69	79	86	7.1
540162	18	33	63	75	87	91	91	92	0.7
540175	10	25	55	76	86	89	89	93	1.0
540182	13	36	61	75	84	88	90	93	0.7
540191	3	12	28	61	79	80	88	94	2.2

Example 38: Efficacy of antisense oligonucleotides targeting human Factor VII in transgenic mice

Transgenic mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for efficacy.

Treatment

Eight groups of 3 transgenic mice each were injected subcutaneously twice a week for 3 weeks with 10 mg/kg, 5 mg/kg, 2.5 mg/kg, or 1.25 mg/kg of ISIS 407935 or ISIS 490279. Another 24 groups of 3 transgenic mice each were subcutaneously twice a week for 3 weeks with 2.5 mg/kg, 1.25 mg/kg, 0.625

mg/kg, or 0.313 mg/kg of ISIS 473589, ISIS 529804, ISIS 534796, ISIS 540162, ISIS 540175, or ISIS 540191. One group of mice was injected subcutaneously twice a week for 3 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

RNA Analysis

5 RNA was extracted from plasma for real-time PCR analysis of Factor VII, using primer probe set RTS2927. The mRNA levels were normalized using RIBOGREEN®. As shown in Table 35, several antisense oligonucleotides achieved significant reduction of human Factor VII over the PBS control. Results are presented as percent inhibition of Factor VII, relative to control. MOE' indicates that the antisense oligonucleotide is a MOE gapmer. 'DMC' indicates that the antisense oligonucleotide comprises deoxy, cEt and MOE modifications. Treatment with newly designed MOE gapmer, ISIS 490279, caused greater reduction in human Factor VII mRNA levels than treatment with ISIS 407935, the MOE gapmer from the earlier publication. Treatment with several of the newly designed DMC oligonucleotides also caused greater reduction in human Factor VII mRNA levels than treatment with ISIS 407935.

Table 35
Percent inhibition of Factor VII mRNA in transgenic mice

ISIS No	Chemistry	Dose (mg/kg/wk)	% inhibition
407935	MOE	20.0	85
		10.0	57
		5.0	45
		2.5	28
490279	MOE	20.0	88
		10.0	70
		5.0	51
		2.5	33
473589	DMC	5.00	80
		2.50	62
		1.25	44
		0.625	25
529804	DMC	5.00	55
		2.50	41
		1.25	0
		0.625	1
534796	DMC	5.00	56
		2.50	41
		1.25	5
		0.625	0
540162	DMC	5.00	97
		2.50	92
		1.25	69
		0.625	78

540175	DMC	5.00	95
		2.50	85
		1.25	65
		0.625	55
540182	DMC	5.00	97
		2.50	83
		1.25	54
		0.625	10
540191	DMC	5.00	91
		2.50	74
		1.25	58
		0.625	34

Protein Analysis

Plasma protein levels of Factor VII were estimated using a Zymutest FVII ELISA kit (Hyphen Bio-Med cat# ARK036A). As shown in Table 36, several antisense oligonucleotides achieved significant reduction of human Factor VII over the PBS control. Results are presented as percent inhibition of Factor VII, relative to control. MOE' indicates that the antisense oligonucleotide is a MOE gapmer. 'DMC' indicates that the antisense oligonucleotide comprises deoxy, cEt and MOE modifications. Treatment with newly designed MOE gapmer, ISIS 490279, caused greater reduction in human Factor VII protein levels than treatment with ISIS 407935, the MOE gapmer from the earlier publication. Treatment with several of the newly designed DMC oligonucleotides also caused greater reduction in human Factor VII protein levels than treatment with ISIS 407935.

Table 36
Percent inhibition of Factor VII plasm protein levels in transgenic mice

ISIS No	Chemistry	Dose (mg/kg/wk)	% inhibition
407935	MOE	20	65
		10	47
		5	0
		2.5	3
490279	MOE	20	91
		10	75
		5	31
		2.5	23
473589	DMC	5	78
		2.5	40
		1.25	6
		0.625	0
529804	DMC	5	50
		2.5	36
		1.25	0

		0.625	8
534796	DMC	5	45
		2.5	26
		1.25	0
		0.625	8
540162	DMC	5	98
		2.5	96
		1.25	78
		0.625	74
540175	DMC	5	93
		2.5	83
		1.25	49
		0.625	24
540182	DMC	5	97
		2.5	71
		1.25	50
		0.625	0
540191	DMC	5	97
		2.5	74
		1.25	46
		0.625	25

Example 39: Effect of ISIS antisense oligonucleotides targeting human Factor VII in cynomolgus monkeys

Cynomolgus monkeys were treated with ISIS antisense oligonucleotides selected from studies described above, including ISIS 407935, ISIS 490279, ISIS 473589, ISIS 529804, ISIS 534796, ISIS 540162, ISIS 540175, ISIS 540182, and ISIS 540191. Antisense oligonucleotide efficacy and tolerability were evaluated. ISIS 407935, from the earlier publication, was included in the study for comparison. The antisense oligonucleotides tested in the study are presented in Table 37. The ‘Sugar Chemistry’ column provides the sugar modifications throughout each oligonucleotide: ‘d’ indicates a 2’-deoxynucleoside, ‘k’ indicates a constrained ethyl (cEt) nucleoside, and ‘e’ indicates a 2’-O-methoxyethyl nucleoside. The ‘Sequence’ column provides the nucleobase sequence for each SEQ ID NO. Some of the human antisense oligonucleotides tested are also cross-reactive with the rhesus genomic sequence (GENBANK Accession No. NW_001104507.1 truncated from nucleotides 691000 to 706000, designated herein as SEQ ID NO: 4). The greater the complementarity between the human oligonucleotide and the rhesus monkey sequence, the more likely the human oligonucleotide can cross-react with the rhesus monkey sequence. ‘Mismatches’ indicate the number of nucleotides between the human oligonucleotide and the rhesus monkey sequence that are mismatched. Mismatches of more than 3 have not been shown. “Start site” indicates the 5’-most nucleotide to which the oligonucleotide is targeted in the rhesus monkey gene sequence.

Table 37
Antisense oligonucleotides selected for the cynomolgous monkey study

Start Site	Mismatches	Sequence	SEQ ID NO	ISIS No	Sugar Chemistry
12908	0	ATGCATGGTGATGCTTCTGA	120	407935	eeeeeddddddddeeeee
12845	0	GCTAAACAACCGCCTT	59	473589	kdkdkdddddddeee
808	0	CCCTCCTGTGCCTGGATGCT	93	490279	eeeeeddddddddeeeee
12909	0	CATGGTGATGCTTCTG	259	529804	kdddddddddkekee
12848	0	AGAGCTAAACAACCGC	254	534796	ekdddddddddkke
2041	2	ACTCCCGGGACACCCA	624	540162	eekdddddddddkke
2058	1				
2075	3				
2108	1				
2125	3				
2142	3				
2159	3				
2175	3				
2191	3				
2208	1				
2225	3				
2258	2				
2292	1				
2309	1				
2324	1				
2358	0				
2358	0	GGACACCCACGCCCCC	637	540175	eekdddddddddkke
2017	2				
2051	0				
2068	3				
2085	1				
2101	3				
2118	3				
2135	1				
2152	2				
2168	1				
2184	1				
2201	1				
2218	3				
2234	3				
2251	1				
2268	2				
2285	0				
2302	1				
2334	3				
2351	1				
2368	3				

2049	2	ACACCCTCGCCTCCGG	644	540182	eekdddddddddkke
2133	3				
2150	2				
2166	3				
2182	3				
2199	3				
2216	3				
2266	3				
2300	3				
2041	2	GCCTCCGGAACACCCA	653	540191	eekdddddddddkke
2075	3				
2125	3				
2142	1				
2191	3				
2208	3				
2258	2				
2292	3				
2309	3				

Treatment

Prior to the study, the monkeys were kept in quarantine for at least a 30-day period, during which the
 5 animals were observed daily for general health. Standard panels of serum chemistry and hematology,
 examination of fecal samples for ova and parasites, and a tuberculosis test were conducted immediately after
 the animals' arrival to the quarantine area. The monkeys were 2-4 years old at the start of treatment and
 weighed between 2 and 4 kg. Ten groups of four randomly assigned male cynomolgus monkeys each were
 injected subcutaneously with ISIS oligonucleotide or PBS using a stainless steel dosing needle and syringe of
 10 appropriate size into one of 4 sites on the back of the monkeys; each site used in clock-wise rotation per dose
 administered. Nine groups of monkeys were dosed four times a week for the first week (days 1, 3, 5, and 7)
 as loading doses, and subsequently once a week for weeks 2-12, with 35 mg/kg of ISIS 407935, ISIS 490279,
 ISIS 473589, ISIS 529804, ISIS 534796, ISIS 540162, ISIS 540175, ISIS 540182, or ISIS 540191. A control
 group of cynomolgus monkeys was injected with PBS subcutaneously thrice four times a week for the first
 15 week (days 1, 3, 5, and 7), and subsequently once a week for weeks 2-12.

During the study period, the monkeys were observed twice daily for signs of illness or distress. Any
 animal experiencing more than momentary or slight pain or distress due to the treatment, injury or illness was
 treated by the veterinary staff with approved analgesics or agents to relieve the pain after consultation with
 the Study Director. Any animal in poor health or in a possible moribund condition was identified for further
 20 monitoring and possible euthanasia. Terminal sacrifice was performed on day 86, approximately 48 hours
 after the final dosing on day 84. The protocols described in the Example were approved by the Institutional
 Animal Care and Use Committee (IACUC).

Necroscopy

For terminal necroscopy on day 86, approximately 48 hours after the final dose, the animals were euthanized by exsanguination while under deep anesthesia. A full macroscopic examination was performed under the general supervision of a pathologist and all lesions were recorded. Of note, treatment with ISIS 407935 was observed to result in ascites in 2 out of 4 monkeys suggesting it is less well tolerated than the other compounds in the study. Specifically, compounds ISIS Nos: 490279, 473589, 540162, 534796, and 540175 did not show any of these findings.

Hepatic Target Reduction*RNA analysis*

On day 86, RNA was extracted from liver tissue for real-time PCR analysis of Factor VII using primer probe set RTS2927. Results are presented as percent inhibition of Factor VII mRNA, relative to PBS control, normalized to RIBOGREEN® or to the house keeping gene, GAPDH. As shown in Table 38, treatment with ISIS antisense oligonucleotides resulted in significant reduction of Factor VII mRNA in comparison to the PBS control.

Table 38

Percent Inhibition of cynomolgous monkey Factor VII mRNA in the cynomolgus monkey liver relative to the PBS control

ISIS No	RTS2927/Ribogreen	RTS2927/GAPDH
407935	90	90
490279	72	66
473589	96	96
529804	90	87
534796	80	78
540162	66	58
540175	68	66
540182	0	0
540191	34	14

Protein levels and activity analysis

Plasma Factor VII levels were measured prior to dosing, and on day 3, day 5, day 7, day 16, day 30, day 44, day 65, and day 86 of treatment. Factor VII activity was measured using Factor VII deficient plasma. Approximately 1.5 mL of blood was collected from all available study animals into tubes containing 3.2% sodium citrate. The samples were placed on ice immediately after collection. Collected blood samples were processed to platelet poor plasma and the tubes were centrifuged at 3,000 rpm for 10 min at 4°C to obtain plasma.

Protein levels of Factor VII were measured by a ZYMUTEST Factor VII elisa kit from Hyphen BioMed (cat# RK036A). The results are presented in Table 39. To measure Factor VII activity, 60 µL of sample plasma was diluted 1/20 in factor diluents buffer and then incubated with 60 µL of PT reagent (PT-Fibrinogen HS, Instrumentation Laboratory Company, USA) and 60 µL of citrated human plasma deficient of Factor VII (George King Bio-Medical Inc., USA) at 37°C for 5 min. Factor VII activity was then determined with ACL-9000 (Instrumentation Laboratory, Italy). The results, in seconds, for Factor VII activity was interpolated on a standard curve of serial dilutions from normal pooled monkey plasma. The results are presented in Table 40, expressed as a percentage reduction compared to the baseline values.

Table 39

Plasma Factor VII protein levels (% reduction compared to the baseline) in the cynomolgus monkey plasma

ISIS No	Day 3	Day 5	Day 7	Day 16	Day 30	Day 44	Day 65	Day 86
407935	21	62	69	82	84	85	84	90
490279	0	29	35	30	38	45	51	58
473589	12	67	85	97	98	98	98	98
529804	19	65	76	87	88	89	90	90
534796	1	46	54	64	64	67	66	70
540162	0	24	26	37	45	49	49	50
540175	0	28	36	38	47	52	55	55
540182	0	17	8	0	0	0	5	0
540191	0	12	4	0	0	4	9	10

Table 40

Plasma Factor VII activity levels (% reduction compared to the baseline) in the cynomolgus monkey plasma

ISIS No	Day 3	Day 5	Day 7	Day 16	Day 30	Day 44	Day 65	Day 86
407935	25	76	80	90	91	87	89	92
490279	0	8	4	31	40	57	56	66
473589	21	78	86	98	97	98	98	98
529804	25	69	81	93	87	92	93	93
534796	5	47	63	76	65	76	74	76
540162	0	0	7	30	26	50	49	51
540175	0	16	36	44	50	67	60	63
540182	0	0	12	5	24	15	0	4
540191	0	13	17	19	30	61	28	32

Tolerability studies

Body and organ weight measurement

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body and organ weights were measured on different days. The data is presented in Table 41. The results indicate that effect of treatment with antisense oligonucleotides on body weights was within the normal range. However, treatment with ISIS 407935 resulted in a 2.2-fold increase in spleen weight, a 2.7-fold increase in liver weight, and a

1.3-fold increase in kidney weight compared to the control, indicating that ISIS 407935 had an effect on organ weights, which was not observed with the newly designed antisense oligonucleotides.

Table 41

Final body weights (grams) in the cynomolgus monkey relative to pre-dose levels

	Day 1	Day 7	Day 21	Day 28	Day 42	Day 63	Day 84
PBS	2651	2634	2672	2685	2694	2755	2767
ISIS 407935	2567	2506	2536	2548	2545	2528	2537
ISIS 490279	2597	2566	2597	2635	2713	2765	2850
ISIS 473589	2606	2618	2656	2657	2692	2734	2777
ISIS 529804	2597	2580	2590	2627	2651	2657	2734
ISIS 534796	2569	2596	2628	2622	2666	2738	2789
ISIS 540162	2715	2747	2743	2755	2799	2758	2934
ISIS 540175	2644	2678	2675	2687	2720	2760	2812
ISIS 540182	2517	2529	2528	2533	2674	2716	2790
ISIS 540191	2590	2598	2661	2686	2750	2833	2938

5

Serum chemistry markers

To evaluate the effect of ISIS oligonucleotides on serum chemistry markers, the monkeys were fasted overnight prior to blood collection. Approximately 1.5 mL of blood was collected into tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 min and then centrifuged at 3,000 rpm for 10 min at room temperature. Serum levels of various markers were measured on day 44 using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co. Japan). Levels of ALT and AST were measured, and the results are presented in Table 42, expressed in IU/L. Serum creatinine, and BUN were similarly measured and also presented in Table 42, expressed in mg/dL. Serum C-reactive protein (CRP) was also similarly measured and is presented in Table 42, expressed as mg/L. Serum albumin was also similarly measured and is presented in Table 42, expressed in g/dL. In monkeys treated with ISIS 407935, there was an elevation in serum BUN, CRP, and creatinine levels, indicating the treatment with ISIS 407935 may have produced deleterious effects on kidney function and an acute stress response. Treatment with the newly designed oligonucleotides produced no changes within these parameters suggesting they have a more favorable safety profile than treatment with ISIS 407935.

20

Table 42

Effect of antisense oligonucleotide treatment on liver function markers in cynomolgus monkey plasma

	ALT (IU/L)	AST (IU/L)	Creatinine (mg/dL)	BUN (mg/dL)	Albumin (g/dL)	CRP (mg/L)
--	------------	------------	--------------------	-------------	----------------	------------

PBS	51	61	0.9	28	4.5	1.6
407935	52	64	1.5	52	4.0	5.2
490279	100	60	1.0	23	4.7	2.3
473589	55	52	1.0	24	4.8	2.7
529804	48	46	1.0	28	4.5	2.1
534796	40	57	1.0	30	4.5	1.6
540162	45	55	1.1	25	4.7	1.3
540175	46	44	0.9	21	4.7	1.1
540182	123	129	0.9	28	4.4	1.5
540191	36	41	1.0	24	4.7	1.6

Urine chemistry markers

To evaluate the effect of ISIS oligonucleotides on kidney function, fresh urine from all animals was collected for urinalysis using a clean cage pan on ice. Food was removed overnight the day before urine collection but water was supplied. Levels of creatinine and total urine protein were measured on day 86 using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). The ratio of total urine protein to creatine was then calculated and the results are presented in Table 43.

The data indicate that most of the newly designed ISIS oligonucleotides did not have any effect on the kidney function outside the expected range. However, treatment with ISIS 407935 resulted in elevated urine protein to creatinine ratio in the monkeys, indicating treatment with ISIS 407935 perturbed kidney function. Hence, treatment with the newly designed oligonucleotides was more tolerable than treatment with ISIS 407935.

Table 43
Total urine protein to creatinine ratio in cynomolgus monkeys

	Protein/creatinine ratio
PBS	0.03
ISIS 407935	0.64
ISIS 490279	0.00
ISIS 473589	0.01
ISIS 529804	0.00
ISIS 534796	0.00
ISIS 540162	0.01
ISIS 540175	0.00
ISIS 540182	0.04
ISIS 540191	0.26

Complement C3 analysis

To evaluate any effect of ISIS oligonucleotides on complement C3 levels, approximately 0.5 mL of blood was collected into tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 min and then centrifuged at 3,000 rpm for 10 min at room temperature to

obtain serum. Complement C3 was measured at week 1, 24 hours after dosing, using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). The data is presented in Table 44, expressed in mg/dL. Treatment with ISIS 407935 resulted in reduced complement C3 levels, indicating treatment with ISIS 407935 may have resulted in repeated complement activation to a greater degree than with the newly designed oligonucleotides.

Table 44
Complement C3 levels in cynomolgus monkeys

	mg/dL
PBS	146
ISIS 407935	92
ISIS 490279	124
ISIS 473589	140
ISIS 529804	137
ISIS 534796	137
ISIS 540162	135
ISIS 540175	121
ISIS 540182	104
ISIS 540191	141

Hematology

To evaluate any effect of ISIS oligonucleotides in cynomolgus monkeys on hematologic parameters, blood samples of approximately 0.5 mL of blood was collected on day 44 from each of the available study animals in tubes containing K₂-EDTA. Samples were analyzed for red blood cell (RBC) count, as well as for platelet count, using an ADVIA120 hematology analyzer (Bayer, USA). The data is presented in Table 45.

Table 45
Complement C3 levels in cynomolgus monkeys

	Platelet count ($\times 10^3/\mu\text{L}$)	RBC count ($\times 10^6/\mu\text{L}$)
PBS	378	6.0
ISIS 407935	367	5.8
ISIS 490279	457	6.0
ISIS 473589	472	5.9
ISIS 529804	343	5.7
ISIS 534796	473	5.8
ISIS 540162	379	5.9
ISIS 540175	445	5.9
ISIS 540182	481	5.7
ISIS 540191	528	5.9

Coagulation

To evaluate any effect of ISIS oligonucleotides on the coagulation cascade, blood samples of approximately 1.0 mL of blood was collected on day 44 from each of the available study animals in tubes containing 3.2% sodium citrate. Plasma samples were obtained after centrifugation at 3,000 rpm for 10 min at room temperature. PT and aPTT were measured using an ACL 9000 coagulation analyzer (Instrumentation Laboratory, Italy). The data is presented in Table 46.

Treatment with ISIS 407935, ISIS 473589 and ISIS 529804 caused an increase in PT, which is an expected outcome due to the reduction in Factor VII protein and activity as a result of antisense inhibition.

Table 46
PT and aPTT (seconds) in cynomolgus monkeys

	PT	aPTT
PBS	10.05	19.48
ISIS 407935	13.05	49.73
ISIS 490279	10.15	19.73
ISIS 473589	21.33	18.38
ISIS 529804	13.88	18.43
ISIS 534796	11.10	18.23
ISIS 540162	10.75	18.00
ISIS 540175	10.50	19.05
ISIS 540182	10.60	22.00
ISIS 540191	10.93	19.30

Example 40: Dose-dependent antisense inhibition of human Factor VII in HepG2 cells

Antisense oligonucleotides (from Example 37) were tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells μ M per well and transfected using electroporation with 0.003 μ M, 0.016 μ M, 0.800 μ M, 4.000 μ M, and 20.000 μ M 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 VII mRNA levels were measured by quantitative real-time PCR. Factor VII primer probe set RTS2927 was used to measure mRNA levels. Factor VII mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN®. Results are presented as percent inhibition of Factor VII expression relative to untreated control cells. As illustrated in Table 47, Factor VII mRNA levels were significantly reduced in a dose-dependent manner with several of the antisense oligonucleotides.

Table 47

Dose-dependent antisense inhibition (%) of Factor VII in HepG2 cells using electroporation

ISIS No	0.003 μ M	0.016 μ M	0.800 μ M	4.000 μ M	20.000 μ M	IC ₅₀ (μ M)
407935	14	27	70	87	96	0.4

473589	15	39	72	89	88	0.3
490279	9	11	47	63	67	2.2
515533	0	13	53	78	85	1.1
515952	7	42	78	92	95	0.3
516066	5	26	45	73	84	1
529459	1	12	53	81	79	1.1
529553	11	13	57	79	91	0.8
529804	3	36	82	89	92	0.4
534796	17	46	76	90	87	0.3
537806	1	9	39	50	70	3.5
540162	27	59	76	86	93	0.1
540175	19	61	76	65	90	0.2
540182	40	66	81	85	89	0.04
540191	27	50	77	81	93	0.2

CLAIMS

1. 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, at least 10, at least 12, at least 14, at least 16, at least 18, at least 19, or at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1381 to 1406 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
2. The compound of claim 1, wherein the modified oligonucleotide consists of 15 to 30, 18 to 24, 19 to 22, or 20 linked nucleosides.
3. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 15128 to 15150 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
4. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2592 to 2607, 2626 to 2641, 2660 to 2675, 2796 to 2811, 2966 to 2981, 3000 to 3015, 3034 to 3049, 3068 to 3083, 3153 to 3168, 3170 to 3185, 3272 to 3287, 3374 to 3389, 3578 to 3593, 3851 to 3866, 3953 to 3968, 4124 to 4139, 4260 to 4275, 4311 to 4326, 4447 to 4462, and 4532 to 4547 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
5. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2592 to 2607, 2626 to 2641, 2660 to 2675, 2796 to 2811, 2966 to 2981, 3000 to 3015, 3034 to 3049, 3068 to 3083, 3153 to 3168, 3170 to 3185, 3272 to 3287, 3374 to 3389, 3578 to 3593, 3851 to 3866, 3953 to 3968, 4124 to 4139, 4260 to 4275, 4311 to 4326, 4447 to 4462, or 4532 to 4547 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

6. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 1387 to 1406, 15128 to 15143, 15192 to 15207, and 15131 to 15146 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
7. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2692 to 2707, 2760 to 2775, 2862 to 2877, 2930 to 2945, 3117 to 3132, 3338 to 3353, 3440 to 3455, 3508 to 3523, 3542 to 3557, 3628 to 3643, 3662 to 3677, 3781 to 3796, 3815 to 3830, 3917 to 3932, 4190 to 4205, 4224 to 4239, 4377 to 4392, and/or 4411 to 4426 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
8. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 3109 to 3124, 3194 to 3209, 3330 to 3345, 3432 to 3447, 3500 to 3515, 3534 to 3549, 3620 to 3635, 3654 to 3669, 3773 to 3788, 4182 to 4197, 4216 to 4231, 4369 to 4384, and/or 4403 to 4418 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
9. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2565 to 2580, 2633 to 2648, 2667 to 2682, 2735 to 2750, 2803 to 2818, 2837 to 2852, 2905 to 2920, 3007 to 3022, 3041 to 3056, 3075 to 3090, 3092 to 3107, 3279 to 3294, 3381 to 3396, 3483 to 3498, 3603 to 3618, 3722 to 3737, 3756 to 3771, 3858 to 3873, 3892 to 3907, 3960 to 3975, 4046 to 4061, 4131 to 4146, 4165 to 4180, 4318 to 4333, and/or 4454 to 4469 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

10. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 2558 to 4600 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
11. 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, at least 10, at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases complementary to an equal length portion of nucleobases 15128 to 15150, 15181 to 15224, 15128 to 15150, 2560 to 2609, 2684 to 2717, or 3103 to 3131 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.
12. The compound of any preceding claim, wherein the modified oligonucleotide consists of 13 to 25, 14 to 25, 15 to 25, or 16 linked nucleosides.
13. The compound of any preceding claim, wherein the nucleobase sequence of the modified oligonucleotide is at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to SEQ ID NO: 1.
14. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 59.
15. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 16, at least 18, at least 19, or at least 20 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 93.
16. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 637.

17. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of any of the nucleobase sequences of SEQ IDNO: 59, 93, 259, 254, 624, 637, 644, or 653.
18. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 14, at least 15, or at least 16 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NO: 21-559.
19. The compound of any preceding claim, consisting of a single-stranded modified oligonucleotide.
20. The compound of any preceding claim, wherein at least one internucleoside linkage is a modified internucleoside linkage.
21. The compound of claim 20, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.
22. The compound of any preceding claim, wherein at least one nucleoside comprises a modified nucleobase.
23. The compound of claim 22, wherein the modified nucleobase is a 5-methylcytosine.
24. The compound of any preceding claim, wherein the modified oligonucleotide comprises at least one modified sugar.
25. The compound of claim 24, wherein the modified sugar is any of a 2'-O-methoxyethyl, a constrained ethyl, or a 3'-fluoro-HNA.
26. The compound of any preceding claim, comprising at least one 2'-O-methoxyethyl nucleoside, a constrained ethyl nucleoside, or a 3'-fluoro-HNA nucleoside.
27. A compound comprising a modified oligonucleotide according to the following formula:
Gks mCds Tks Ads Aks Ads mCds Ads Ads mCds mCds Gds mCds mCds Tes Te; wherein,
each nucleobase is indicated according to the following:
A = adenine

T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

28. A compound consisting of a modified oligonucleotide according to the following formula:

Gks mCds Tks Ads Aks Ads mCds Ads Ads mCds mCds Gds mCds mCds Tes Te; wherein,
each nucleobase is indicated according to the following:

A = adenine

T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

29. A compound comprising of a modified oligonucleotide according to the following formula:

mCes mCes mCes Tes mCes mCds Tds Gds Tds Gds mCds mCds Tds Gds Gds Aes Tes Ges mCes Te;
wherein,

each nucleobase is indicated according to the following:

A = adenine

T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

30. A compound consisting of a modified oligonucleotide according to the following formula:
mCes mCes mCes Tes mCes mCds Tds Gds Tds Gds mCds mCds Tds Gds Gds Aes Tes Ges mCes Te;
wherein,

each nucleobase is indicated according to the following:

A = adenine

T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

31. A compound comprising of a modified oligonucleotide according to the following formula:
Ges Ges Aks mCds Ads mCds mCds mCds Ads mCds Gds mCds mCds mCks mCks mCe; wherein,

each nucleobase is indicated according to the following:

A = adenine

T = thymine

G = guanine;

mC = 5-methylcytosine; wherein

each sugar moiety is indicated according to the following:

k = cEt;

d = 2'-deoxyribose;

e = 2'-MOE; wherein

each internucleoside linkage is indicated according to the following:

s = phosphorothioate.

32. A compound consisting of a modified oligonucleotide according to the following formula:
Ges Ges Aks mCds Ads mCds mCds mCds Ads mCds Gds mCds mCds mCks mCks mCe; wherein,
each nucleobase is indicated according to the following:
A = adenine
T = thymine
G = guanine;
mC = 5-methylcytosine; wherein
each sugar moiety is indicated according to the following:
k = cEt;
d = 2'-deoxyribose;
e = 2'-MOE; wherein
each internucleoside linkage is indicated according to the following:
s = phosphorothioate.
33. A composition comprising a compound according to any of claims 1-32 or a salt thereof and a pharmaceutically acceptable carrier or diluent.
34. A compound according to any of claims 1-32 or a composition according to claim 33, for use in therapy.
35. The compound or composition according to claim 34, for use in treating, preventing, or slowing progression of a thromboembolic complication.
36. The compound or composition according to claim 34, for use in treating, preventing, or slowing progression of a hyperproliferative disorder.
37. The compound or composition according to claim 34, for use in treating, preventing, or slowing progression of an inflammatory condition.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/25381

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/11; C07H 21/04 (2013.01)

USPC - 514/44A; 536/24.5

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC- 514/44A; 536/24.5

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

USPC- 435/375, 514/44R; 536/23.1

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

PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google Patents; Google Scholar: Factor VII, antisense, silencing, interference, dsRNA, RNAi, shRNA

GenCore 6.4.1: SEQ ID NO: 23

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0298417 A1 (Freier, et al.) 25 November 2010 (25.11.2010) para [0328], [0330], Table 7, SEQ ID NO: 395	1, 2, 12

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

05 June 2013 (05.06.2013)

Date of mailing of the international search report

18 JUN 2013

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/25381

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 13, 19-26, 33-37
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

Group I+: claims 1-12, 14-18, 27-32 drawn to 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, at least 10, at least 12, at least 14, at least 16, at least 18, at least 19, or at least 20 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1. The first invention (claims 1, 2, 12) is restricted to SEQ ID NO:23, the first specifically claimed oligonucleotide complementary to a portion of nucleobases 1381-1406 (Table 1, pg 58). Applicant is invited to elect an additional SEQ ID NO to be searched, by paying additional fee per each sequence elected. Exact claims to be searched will depend on the election. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group will result in only the first claimed invention to be searched.

***** See Supplemental Sheet to continue *****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2, 12, restricted to SEQ ID NO:23

Remark on Protest

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 13/25381

***** Supplemental Sheet *****

In Continuation of Box III. Observations where unity of invention is lacking:

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

Special Technical Features

The special technical feature of each invention of Group I+ is the specific nucleotide sequence.

Common Technical Features

The inventions of Group I+ share the technical feature of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and comprising a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases at least 90% complementary to an equal length portion of SEQ ID NO: 1. However, this shared technical feature does not represent a contribution over prior art as being anticipated by WO 2009/061851 A2 to CROSBY, et al. (14 May 2009) (hereinafter "Crosby") disclosing, in the context of modulation of factor 7 expression (Abstract), a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides and comprising a nucleobase sequence at least 90% complementary to an equal length portion of SEQ ID NO: 1, e.g., at least 8 contiguous nucleobases (15185-15200) SEQ ID NO: 257 (pg 46, Table 1, SEQ ID NO: 53, GGTGATGCTTCTGAATTGTC).

Further, the inventions do not share a special technical feature, because the claimed SEQ ID NO: 1 was known in the art at the time of the invention, as evidenced by Crosby (Crosby, SEQ ID NO: 1, 100% identity), and therefore no significant structural similarities can readily be ascertained among the nucleotide sequences. Without a shared special technical feature, the inventions lack unity with one another.

As the common feature of at least 8 contiguous nucleobases at least 90% complementary to an equal length portion of SEQ ID NO: 1 was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions of Group I+.

Therefore, the inventions of Group I+ lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.