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(54) **Transztiretin expressziójának módosítása**

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(56) References cited:
WO-A1-2010/017509 **WO-A2-2010/048228**
US-A1- 2005 244 869

- **BENSON MERRILL D ET AL: "Targeted suppression of an amyloidogenic transthyretin with antisense oligonucleotides", MUSCLE & NERVE, JOHN WILEY & SONS INC, US, vol. 33, no. 5, 18 January 2006 (2006-01-18), pages 609-618, XP009160090, ISSN: 0148-639X**
- **SEKIJIMA Y ET AL: "Pathogenesis of and Therapeutic Strategies to Ameliorate the Transthyretin Amyloidoses", CURRENT PHARMACEUTICAL DESIGN, BENTHAM SCIENCE PUBLISHERS LTD, NL, vol. 14, no. 30, 2008, pages 3219-3230, XP008121491, ISSN: 1873-4286**
- **KUROSAWA T ET AL: "Selective silencing of a mutant transthyretin allele by small interfering RNAs", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 337, no. 3, 3 October 2005 (2005-10-03), pages 1012-1018, XP027218514, ISSN: 0006-291X, DOI: 10.1016/J.BBRC.2005.09.142 [retrieved on 2005-10-18]**
- **TASAKI M ET AL: "siRNA therapy for TTR-related ocular amyloidosis", AMYLOID: THE INTERNATIONAL JOURNAL OF EXPERIMENTAL AND CLINICAL INVESTIGATION, PARTHENON PUBLISHING GROUP, LANCS, GB, vol. 17, no. suppl. 1, 21 April 2010 (2010-04-21), pages 52-53, XP009172275, ISSN: 1350-6129**
- **BENSON MERRILL D ET AL: "The molecular biology and clinical features of amyloid neuropathy", MUSCLE & NERVE, WILEY, US, vol. 36, no. 4, 6 June 2007 (2007-06-06), pages 411-423, XP009160091, ISSN: 0148-639X, DOI: 10.1002/MUS.20821 [retrieved on 2007-06-06]**

EP 2 563 920 B1

Description

FIELD OF THE INVENTION

[0001] Provided herein are compounds and compositions for reducing expression of transthyretin mRNA and protein in an animal. Such compounds and compositions are useful, for example, to treat, prevent, or ameliorate transthyretin amyloidosis.

BACKGROUND OF THE INVENTION

[0002] Transthyretin (TTR), (also known as prealbumin, hyperthytoxinemia, dysprealbuminemic, thyroxine; senile systemic amyloidosis, amyloid polyneuropathy, amyloidosis I, PALB; dystransthyretinemic, HST2651; TBPA; dysprealbuminemic euthyroidal hyperthyroxinemia) is a serum/plasma and cerebrospinal fluid protein responsible for the transport of thyroxine and retinol (Sakaki et al, Mol Biol Med. 1989, 6:161-8). Structurally, TTR is a homotetramer; point mutations and misfolding of the protein leads to deposition of amyloid fibrils and is associated with disorders, such as senile systemic amyloidosis (SSA), familial amyloid polyneuropathy (FAP), and familial amyloid cardiopathy (FAC).

[0003] TTR is synthesized primarily by the liver and the choroid plexus of the brain and, to a lesser degree, by the retina in humans (Palha, Clin Chem Lab Med, 2002, 40, 1292-1300). Transthyretin that is synthesized in the liver is secreted into the blood, whereas transthyretin originating in the choroid plexus is destined for the CSF. In the choroid plexus, transthyretin synthesis represents about 20% of total local protein synthesis and as much as 25% of the total CSF protein (Dickson et al., J Biol Chem, 1986, 261, 3475-3478).

[0004] With the availability of genetic and immunohistochemical diagnostic tests, patients with TTR amyloidosis have been found in many nations worldwide. Recent studies indicate that TTR amyloidosis is not a rare endemic disease as previously thought, and may affect as much as 25% of the elderly population (Tanskanen et al, Ann Med. 2008;40(3):232-9).

[0005] At the biochemical level, TTR was identified as the major protein component in the amyloid deposits of FAP patients (Costa et al, Proc. Natl. Acad. Sci. USA 1978, 75:4499-4503) and later, a substitution of methionine for valine at position 30 of the protein was found to be the most common molecular defect causing the disease (Saraiva et al, J. Clin. Invest. 1984, 74: 104-119). In FAP, widespread systemic extracellular deposition of TTR aggregates and amyloid fibrils occurs throughout the connective tissue, particularly in the peripheral nervous system (Sousa and Saraiva, Prog. Neurobiol. 2003, 71: 385-400). Following TTR deposition, axonal degeneration occurs, starting in the unmyelinated and myelinated fibers of low diameter, and ultimately leading to neuronal loss at ganglionic sites.

[0006] The compounds and treatment methods described herein provide significant advantages over the treatments options currently available for TTR related disorders. TTR amyloidosis typically lead to death within ten years, and until recently, was considered incurable. Liver transplantation is an effective means of replacing the disease-associated allele by a wild type (WT) allele in familial cases because the liver is typically the source of amyloidogenic TTR. While liver transplantation is effective as a form of gene therapy it is not without its problems. Transplantation is complicated by the need for invasive surgery for the recipient and the donor, long-term post-transplantation immunosuppressive therapy, a shortage of donors, its high cost, and the large number of TTR amyloidosis patients that are not good candidates because of their disease progression. Unfortunately, cardiac amyloidosis progresses in some familial patients even after liver transplantation because WT TTR often continues to deposit. Central nervous system (CNS) deposition of TTR is also not relieved by transplantation owing to its synthesis by the choroid plexus. Transplantation is not a viable option for the most prevalent TTR disease, senile systemic amyloidosis (SSA), affecting approximately 25% of those over 80 due to the deposition of WT TTR.

[0007] Antisense technology is emerging as an effective means for reducing the expression of specific gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of TTR expression (See U.S. Patent Publication Nos. 2008/0039418 and 2007/0299027.

[0008] WO 2010/017509 discloses compounds, compositions and methods for modulating the expression of transthyretin in the brain.

[0009] Benson et al. (2006) Muscle & Nerve 33(5):609-618 describes targeted suppression of an amyloidogenic transthyretin with antisense oligonucleotides.

[0010] Sekijima et al. (2008) Current Pharmaceutical Design 14(30):3219-3230 discusses the pathogenesis of and therapeutic strategies to ameliorate the transthyretin amyloidoses.

[0011] Kurosawa et al. (2005) Biochemical and Biophysical Research Communications 337(3):1012-1018 relates to selective silencing of a mutant transthyretin allele by small interfering RNAs.

[0012] Tasaki et al. (2010) Amyloid: The International Journal of Experimental and Clinical Investigation 17(Suppl. 1):52-53 relates to siRNA therapy for TTR-related ocular amyloidosis.

[0013] Benson et al. (2007) Muscle & Nerve 36(4):411-423 is a review that addresses the molecular biology and

clinical features of amyloid neuropathy.

[0014] WO 2010/048228 relates to compositions and methods for inhibiting expression of transthyretin.

[0015] The present invention provides compositions for modulating transthyretin expression. Antisense compounds for modulating expression of transthyretin are disclosed in the aforementioned published patent applications. However, there remains a need for additional such compounds.

SUMMARY OF THE INVENTION

[0016] The invention provides a compound comprising a modified oligonucleotide having a nucleobase sequence consisting of the 20 linked nucleosides recited in SEQ ID NO: 80.

[0017] The invention also provides a composition comprising the compound of the invention or a salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

[0018] The invention also provides a compound of the invention or a composition of the invention for use in treating transthyretin amyloidosis in a human.

SUMMARY OF THE DISCLOSURE

[0019] Disclosed herein are methods, compounds, and compositions for modulating expression of transthyretin (TTR) mRNA and protein. In certain embodiments, compounds useful for modulating expression of TTR mRNA and protein are antisense compounds. In certain embodiments, the antisense compounds are antisense oligonucleotides.

[0020] In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, TTR mRNA levels are reduced. In certain embodiments, TTR protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

[0021] Disclosed herein are methods, compounds, and compositions for modulating expression of transthyretin and treating, preventing, delaying or ameliorating transthyretin amyloidosis and or a symptom thereof. In certain embodiments are methods, compounds, and compositions for modulating expression of transthyretin and treating, preventing, delaying or ameliorating transthyretin amyloid disease or transthyretin amyloidosis or transthyretin related amyloidosis (e.g., hereditary TTR amyloidosis, leptomeningeal amyloidosis, transthyretin amyloid polyneuropathy, familial amyloid polyneuropathy, familial amyloid cardiomyopathy, or senile systemic amyloidosis).

[0022] In certain embodiments, an animal at risk for transthyretin amyloidosis is treated by administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides, wherein the modified oligonucleotide is complementary to a transthyretin nucleic acid as shown in SEQ ID NO: 1 or SEQ ID NO: 2; or a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 8 to 80 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 a nucleobase sequence selected from any one of nucleobase sequences recited in SEQ ID NOs: 25, 80, 86, 87, 115, 120, 122, or 124.

[0023] In certain embodiments, an animal having transthyretin amyloidosis is treated by administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein the modified oligonucleotide is 100% complementary to a transthyretin nucleic acid as shown in SEQ ID NO: 1 or SEQ ID NO: 2; or a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides and having the nucleobase sequence recited in SEQ ID NO: 80.

[0024] In certain embodiments, an animal having transthyretin amyloidosis is treated by administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein the modified oligonucleotide is 100% complementary to a transthyretin nucleic acid as shown in SEQ ID NO: 1; and wherein the compound comprises a modified oligonucleotide consisting of 20 linked nucleosides having the nucleobase sequence recited in SEQ ID NO: 80.

[0025] In certain embodiments, an animal having transthyretin amyloidosis is treated by administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein the modified oligonucleotide is 100% complementary to a transthyretin nucleic acid as shown in SEQ ID NO: 1; wherein the compound comprises a modified oligonucleotide consisting of 20 linked nucleosides having the nucleobase sequence recited in SEQ ID NO: 80; and wherein the modified oligonucleotides has a gap segment of 10 linked deoxynucleosides between two wing segments that independently have 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 nucleoside is a 2'-substituted nucleoside. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside.

[0026] In certain embodiments, modulation can occur in a cell, tissue, organ or organism. In certain embodiments, the cell, tissue or organ is in an animal. In certain embodiments, the animal is a human. In certain embodiments, transthyretin

mRNA levels are reduced. In certain embodiments, transthyretin protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

[0027] Also disclosed are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions related to transthyretin amyloidosis. In certain embodiments, such diseases, disorders, and conditions are transthyretin amyloidosis related diseases disorders or conditions.

[0028] In certain embodiments, methods of treatment include administering a TTR antisense compound to an individual in need thereof. In certain embodiments, methods of treatment include administering a TTR antisense oligonucleotide to an individual in need thereof.

[0029] In certain embodiments, methods of treatment include administering a transthyretin antisense oligonucleotide and an additional therapy to an individual in need thereof.

DETAILED DESCRIPTION OF THE INVENTION

[0030] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

[0031] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[0032] 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.

[0033] Unless otherwise indicated, the following terms have the following meanings:

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

[0034] "2'-O-methoxyethyl nucleotide" means a nucleotide comprising a 2'-O-methoxyethyl modified sugar moiety.

[0035] "5-methylcytosine" means a cytosine modified with a methyl group attached to the 5' position. A 5-methylcytosine is a modified nucleobase.

[0036] "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 transthyretin is an active pharmaceutical agent.

[0037] "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.

[0038] "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.

[0039] "Administering" means providing a pharmaceutical agent to an individual, and includes, but is not limited to administering by a medical professional and self-administering.

[0040] "Amelioration" refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

[0041] "Amyloidosis" is a group of diseases or disorders resulting from abnormal protein (amyloid or amyloid fibril) deposits in various body tissues. The amyloid proteins may either be deposited in one particular area of the body (localized amyloidosis) or they may be deposited throughout the body (systemic amyloidosis). There are three types of systemic amyloidosis: primary (AL), secondary (AA), and familial (ATTR). Primary amyloidosis is not associated with any other diseases and is considered a disease entity of its own. Secondary amyloidosis occurs as a result of another illness. Familial Mediterranean Fever is a form of familial (inherited) amyloidosis.

[0042] "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.

[0043] "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.

[0044] "Antisense compound" means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

[0045] "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.

[0046] "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.

[0047] "Bicyclic sugar" means a furosyl ring modified by the bridging of two non-geminal ring atoms. A bicyclic sugar is a modified sugar.

[0048] "Bicyclic nucleic acid" or "BNA" refers to a nucleoside or nucleotide wherein the furanose portion of the nucleoside or nucleotide includes a bridge connecting two carbon atoms on the furanose ring, thereby forming a bicyclic ring system.

[0049] "Cap structure" or "terminal cap moiety" means chemical modifications, which have been incorporated at either terminus of an antisense compound.

[0050] "Central nervous system (CNS)" refers to the vertebrate nervous system which is enclosed in meninges. It contains the majority of the nervous system, and consists of the brain (in vertebrates which have brains), and the spinal cord. The CNS is contained within the dorsal cavity, with the brain within the cranial cavity, and the spinal cord in the spinal cavity. The brain is also protected by the skull, and the spinal cord is, in vertebrates, also protected by the vertebrae.

[0051] "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.

[0052] "Chimeric antisense compound" means an antisense compound that has at least two chemically distinct regions.

[0053] "Choroid plexus" is the area on the ventricles of the brain where cerebrospinal fluid (CSF) is produced.

[0054] "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.

[0055] "Complementarity" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

[0056] "Contiguous nucleobases" means nucleobases immediately adjacent to each other.

[0057] "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.

[0058] "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.

[0059] "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.

[0060] "Familial amyloidosis" or "hereditary amyloidosis" is a form of inherited amyloidosis.

[0061] "Familial amyloid polyneuropathy" or "FAP" is a neurodegenerative genetically transmitted disorder, characterized by systemic depositions of amyloid variants of transthyretin proteins, causing progressive sensory and motorial polyneuropathy.

[0062] "Fully complementary" or "100% complementary" means each nucleobase of a nucleobase sequence of a first nucleic acid has a complementary nucleobase in a second nucleobase sequence of 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.

[0063] "Gapmer" means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as a "gap segment" and the external regions may be referred to as "wing segments."

[0064] "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.

[0065] "Hereditary transthyretin (TTR) amyloidosis" is a systemic disease caused by mutations in transthyretin, a plasma transport protein for thyroxine and vitamin A. It is most frequently associated with peripheral neuropathy and restrictive cardiomyopathy, but amyloid deposits in blood vessel walls and connective tissue structures throughout the body often cause dysfunction of other organ systems. Gastrointestinal motility abnormalities are common in this disease with constipation, diarrhea and early satiety from delayed gastric-emptying. Connective tissue deposits of amyloid in the wrist may cause carpal tunnel syndrome. Amyloid deposits in spinal blood vessels and surrounding structures cause spinal stenosis with symptoms of claudication.

[0066] "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.

[0067] "Immediately adjacent" means there are no intervening elements between the immediately adjacent elements.

[0068] "Individual" means a human or non-human animal selected for treatment or therapy.

[0069] "Intracerebroventricular administration" or "cerebral intraventricular administration" or "cerebral ventricular administration" means administration through injection or infusion into the ventricular system of the brain.

[0070] "Intraperitoneal administration" means administration to the peritoneal cavity.

[0071] "Intrathecal administration" means administration through injection or infusion into the cerebrospinal fluid bathing the spinal cord and brain.

[0072] "Intravenous administration" means administration into a vein.

[0073] "Intraventricular administration" means administration into the ventricles of either the brain or heart.

[0074] "Internucleoside linkage" refers to the chemical bond between nucleosides. "Leptomeningeal" means having to do with the leptomeninges, the two innermost layers of tissues that cover the brain and spinal cord. "Leptomeningeal amyloidosis" refers to amyloidosis of the leptomeninges resulting from transthyretin amyloid deposition within the leptomeninges.

[0075] "Linked nucleosides" means adjacent nucleosides which are bonded together.

[0076] "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.

[0077] "Modified internucleoside linkage" refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

[0078] "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).

[0079] "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.

[0080] "Modified oligonucleotide" means an oligonucleotide comprising at least one modified nucleotide.

[0081] "Modified sugar" refers to a substitution or change from a natural sugar.

[0082] "Motif" means the pattern of chemically distinct regions in an antisense compound.

[0083] "naturally occurring internucleoside linkage" means a 3' to 5' phosphodiester linkage.

[0084] "Natural sugar moiety" means a sugar found in DNA (2'-H) or RNA (2'-OH).

[0085] "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). A nucleic acid may also comprise a combination of these elements in a single molecule.

[0086] "nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

[0087] "nucleobase sequence" means the order of contiguous nucleobases independent of any sugar, linkage, or nucleobase modification.

[0088] "Nucleoside" means a nucleobase linked to a sugar.

[0089] "Nucleotide" means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

[0090] "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.

[0091] "Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

[0092] "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. intracerebral administration, intrathecal adminis-

tration, intraventricular administration, ventricular administration, intracerebroventricular administration, cerebral intraventricular administration or cerebral ventricular administration. Administration can be continuous, or chronic, or short or intermittent.

[0093] "Peptide" means a molecule formed by linking at least two amino acids by amide bonds. Peptide refers to polypeptides and proteins.

[0094] "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.

[0095] "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.

[0096] "Phosphorothioate linkage" means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage is a modified internucleoside linkage.

[0097] "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.

[0098] "Prevent" refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to indefinitely. Prevent also means reducing risk of developing a disease, disorder, or condition.

[0099] "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.

[0100] "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.

[0101] "Single-stranded oligonucleotide" means an oligonucleotide which is not hybridized to a complementary strand.

[0102] "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.

[0103] "Subcutaneous administration" means administration just below the skin.

[0104] "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.

[0105] "Target nucleic acid," "target RNA," and "target RNA transcript" all refer to a nucleic acid capable of being targeted by antisense compounds.

[0106] "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.

[0107] "Therapeutically effective amount" means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

[0108] "Transthyretin-specific inhibitor" or "Transthyretin inhibitor" means any compound capable of decreasing transthyretin mRNA or protein expression. Examples of such compounds include a nucleic acid, a peptide, an antibody, or a histone deacetylase inhibitor.

[0109] "Transthyretin specific modulator" or "transthyretin modulator" means any compound capable of increasing or decreasing transthyretin mRNA or protein expression.

[0110] "Transthyretin-related amyloidosis" or "transthyretin amyloidosis" or "Transthyretin amyloid disease", as used herein, is any pathology or disease associated with dysfunction or dysregulation of transthyretin that result in formation of transthyretin-containing amyloid fibrils. Transthyretin amyloidosis includes, but is not limited to, hereditary TTR amyloidosis, leptomeningeal amyloidosis, familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy, familial oculoleptomeningeal amyloidosis, senile cardiac amyloidosis, or senile systemic amyloidosis.,

[0111] "Treat" refers to administering a pharmaceutical composition to effect an alteration or improvement of a disease, disorder, or condition.

[0112] "Unmodified nucleotide" means a nucleotide composed of naturally occurring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. β-D-ribonucleosides) or a DNA nucleotide (i.e. β-D-deoxyribonucleoside).

Certain Embodiments

[0113] Certain embodiments of the disclosure are methods, compounds, and compositions for inhibiting transthyretin expression.

[0114] Certain embodiments disclose antisense compounds targeted to a transthyretin nucleic acid. In certain embodiments, the transthyretin nucleic acid is any of the sequences set forth in GENBANK Accession No. NM_000371.2 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_010966.10 truncated from nucleotides 2009236 to 2017289 (incorporated herein as SEQ ID NO: 2); exons 1-4 extracted from the rhesus monkey genomic sequence GENBANK Accession No. NW_001105671.1, based on similarity to human exons; and GENBANK Accession No. NW_001105671.1 truncated from nucleotides 628000 to 638000 (incorporated herein as SEQ ID NO: 4).

[0115] Certain embodiments disclose compounds comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides wherein the linked nucleosides comprise at least 8 contiguous nucleobases of a sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 25, 80, 86, 87, 115, 120, 122, and 124. In certain embodiments, the modified oligonucleotide comprises at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases of a sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 25, 80, 86, 87, 115, 120, 122, and 124.

[0116] Certain embodiments of the disclosure are compounds comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides wherein the linked nucleosides comprise at least 8 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80. In certain embodiments, the modified oligonucleotide comprises at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases of a sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 80.

[0117] Certain embodiments of the disclosure are compounds comprising a modified oligonucleotide consisting of 12 to 50 linked nucleosides wherein the linked nucleosides comprise at least 8 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80. In certain embodiments, the modified oligonucleotide comprises at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases of a sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 80.

[0118] Certain embodiments of the disclosure are compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides wherein the linked nucleosides comprise at least 8 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80. In certain embodiments, the modified oligonucleotide comprises at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases of a sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 80.

[0119] Certain embodiments of the disclosure are compounds comprising a modified oligonucleotide consisting of 15 to 25 linked nucleosides wherein the linked nucleosides comprise at least 8 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80. In certain embodiments, the modified oligonucleotide comprises at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases of a sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 80.

[0120] Certain embodiments of the disclosure are compounds comprising a modified oligonucleotide consisting of 18 to 21 linked nucleosides wherein the linked nucleosides comprise at least 8 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80. In certain embodiments, the modified oligonucleotide comprises at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases of a sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 80.

[0121] Certain embodiments of the disclosure are compounds comprising a modified oligonucleotide consisting of 20 to 30 linked nucleosides wherein the linked nucleosides comprise at least 8 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80. In certain embodiments, the modified oligonucleotide comprises at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80.

[0122] In certain embodiments, the compound comprises a modified oligonucleotide consisting of 20 linked nucleosides recited in SEQ ID NO: 80.

[0123] In certain embodiments, the modified oligonucleotide consists of a single-stranded modified oligonucleotide.

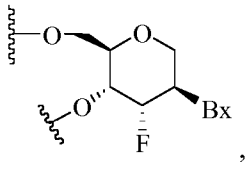
[0124] In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides.

[0125] In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 1,2, or 4. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 95% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 1,2, or 4. In certain embodiments, the modified oligonucleotide is at least 99% complementary over its entire length to SEQ ID NO: 1,2, or 4. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is 100% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 1, 2, or 4.

[0126] In certain embodiments, the compound has at least one modified internucleoside linkage. In certain embodiments, the internucleoside linkage is a phosphorothioate internucleoside linkage.

[0127] In certain embodiments, the compound has at least one nucleoside comprising a modified sugar. In certain embodiments, the at least one modified sugar is a bicyclic sugar. In certain embodiments, the at least one bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' bridge. In certain embodiments, the at least one modified sugar comprises a 2'-O-methoxyethyl.

[0128] In certain embodiments, the compound comprises at least one at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring. In certain embodiments, the at least one tetrahydropyran modified nucleoside has the structure:



,

wherein Bx is an optionally protected heterocyclic base moiety.

[0129] In certain embodiments, the compound has at least one nucleoside comprising a modified nucleobase. In certain embodiments, the modified nucleobase is a 5 -methylcytosine.

[0130] In certain embodiments, the modified oligonucleotide of the compound comprises:

(i) a gap segment consisting of linked deoxynucleosides;
(ii) a 5' wing segment consisting of linked nucleosides;
(iii) a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

[0131] In certain embodiments, the modified oligonucleotide of the compound comprises:

(i) a gap segment consisting of ten linked deoxynucleosides;
(ii) a 5' wing segment consisting of five linked nucleosides;
(iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

[0132] In certain embodiments, the modified oligonucleotide of the compound comprises:

(i) a gap segment consisting of eight linked deoxynucleosides;
(ii) a 5' wing segment consisting of six linked nucleosides;
(iii) a 3' wing segment consisting of six linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

[0133] In certain embodiments, the modified oligonucleotide of the compound comprises:

(i) a gap segment consisting of eight linked deoxynucleosides;
(ii) a 5' wing segment consisting of five linked nucleosides;
(iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

[0134] In certain embodiments, the modified oligonucleotide of the compound comprises:

(i) a gap segment consisting of ten linked deoxynucleosides;
(ii) a 5' wing segment consisting of five linked nucleosides;
(iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing

segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage; and wherein the nucleobase sequence comprises at least 8 contiguous nucleobases of the nucleobase sequence recited in SEQ ID NO: 80.

[0135] In certain embodiments, the modified oligonucleotide of the compound comprises:

(i) a gap segment consisting of ten linked deoxynucleosides;
(ii) a 5' wing segment consisting of five linked nucleosides;
(iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage; and wherein the nucleobase sequence is recited in SEQ ID NO: 80.

[0136] Certain embodiments disclose a composition comprising a compound as described herein, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 25, 80, 86, 87, 115, 120, 122, and 124 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

[0137] Certain embodiments disclose a composition comprising a compound as described herein, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of the nucleobase sequences recited in SEQ ID NO: 80 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

[0138] Certain embodiments disclose a composition comprising a compound as described herein, wherein the viscosity level is less than 40 cP. In certain embodiments, the composition has a viscosity level less than 15 cP. In certain embodiments, the composition has a viscosity level less than 12 cP. In certain embodiments, the composition has a viscosity level less than 10 cP.

[0139] Certain embodiments of the disclosure are methods of treating, preventing, or ameliorating transthyretin amyloidosis.

[0140] Certain embodiments disclose methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 25, 80, 86, 87, 115, 120, 122, and 124.

[0141] Certain embodiments disclose methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a compound or modified oligonucleotide consisting 12 to 30 linked nucleosides, wherein the linked nucleosides comprise at least an 8 contiguous nucleobase portion complementary to an equal length portion within the region selected from nucleotides 501-535 or 580-608 of SEQ ID NO: 1.

[0142] Certain embodiments disclose methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 80.

[0143] Certain embodiments disclose methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a compound or modified oligonucleotide consisting 12 to 30 linked nucleosides, wherein the linked nucleosides comprise at least an 8 contiguous nucleobase portion complementary to an equal length portion within the region selected from nucleotides 508-527 of SEQ ID NO: 1.

[0144] In certain embodiments, the animal is a human.

[0145] In certain embodiments, the administering prevents, treats, ameliorates, or slows progression of transthyretin amyloidosis as described herein.

[0146] In certain embodiments, the compound is co-administered with a second agent.

[0147] In certain embodiments, the compound and the second agent are administered concomitantly.

[0148] In certain embodiments, the administering is parenteral administration. In certain embodiments, the parenteral administration is subcutaneous administration. In certain embodiments, the formulation for administering is the compound in saline. In certain embodiments, the compound comprises a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 25, 80, 86, 87, 115, 120, 122, and 124

or a salt thereof and saline. In certain embodiments, the formulation does not include any stabilizing agents or additional stabilizing agents including lipid agents.

[0149] In certain embodiments, the administering is parenteral administration. In certain embodiments, the parenteral administration is intracranial administration. In certain embodiments, the intracranial administration is intracerebral, intrathecal, intraventricular, ventricular, intracerebroventricular, cerebral intraventricular or cerebral ventricular administration.

[0150] Certain embodiments of the disclosure are methods to reduce transthyretin mRNA or protein expression in an animal comprising administering to the animal a compound or composition as described herein to reduce transthyretin mRNA or protein expression in the animal. In certain embodiments, the animal is a human. In certain embodiments, reducing transthyretin mRNA or protein expression prevents, treats, ameliorates, or slows progression of transthyretin amyloidosis.

[0151] Certain embodiments of the disclosure are methods for treating a human with a transthyretin related disease comprising identifying the human with the disease and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of restlessness, lack of coordination, nystagmus, spastic paraparesis, lack of muscle coordination, impaired vision, insomnia, unusual sensations, myoclonus, blindness, loss of speech, Carpal tunnel syndrome, seizures, subarachnoid hemorrhages, stroke and bleeding in the brain, hydrocephalus, ataxia, and spastic paralysis, coma, sensory neuropathy, parathesia, hypesthesia, motor neuropathy, autonomic neuropathy, orthostatic hypotension, cyclic constipation, cyclic diarrhea, nausea, vomiting, reduced sweating, impotence, delayed gastric emptying, urinary retention, urinary incontinence, progressive cardiopathy, fatigue, shortness of breath, weight loss, lack of appetite, numbness, tingling, weakness, enlarged tongue, nephrotic syndrome, congestive heart failure, dyspnea on exertion, peripheral edema, arrhythmias, palpitations, light-headedness, syncope, postural hypotension, peripheral nerve problems, sensory motor impairment, lower limb neuropathy, upper limb neuropathy, hyperalgesia, altered temperature sensation, lower extremity weakness, cachexia, peripheral edema, hepatomegaly, purpura, diastolic dysfunction, premature ventricular contractions, cranial neuropathy, diminished deep tendon reflexes, amyloid deposits in the corpus vitreum, vitreous opacity, dry eyes, glaucoma, scalloped appearance in the pupils, swelling of the feet due to water retention. In certain embodiments, the symptom is a cognitive symptom selected from the group consisting of impaired memory, impaired judgment, and thinking, impaired planning, impaired flexibility, impaired abstract thinking, impaired rule acquisition, impaired initiation of appropriate actions, impaired inhibition of inappropriate actions, impaired short-term memory, impaired long-term memory, paranoia, disorientation, confusion, hallucination and dementia. In certain embodiments, the symptom is a psychiatric symptom selected from the group consisting of dementia; anxiety, depression, blunted affect, egocentrisms, aggression, compulsive behavior, irritability, personality changes, including, impaired memory, judgment, and thinking and suicidal ideation.

[0152] Further embodiments of the disclosure are methods of treating a human with transthyretin amyloidosis leading to cardiac amyloidosis and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of congestive heart failure, cardiomegaly, dyspnea on exertion, peripheral edema, arrhythmias, palpitations, lightheadedness, syncope, deposition in the subendothelium of the peripheral vasculature can lead to severe postural hypotension, diastolic dysfunction, heart block, premature ventricular contractions, and various tachyarrhythmias.

[0153] Further embodiments of the disclosure are methods of treating a human with transthyretin amyloidosis leading to peripheral neuropathic disorders and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of peripheral nerve problems, sensorimotor impairment, lower-limb neuropathy, upper-limb neuropathy, hyperalgesia, altered temperature sensation, lower extremity weakness, pain, autonomic dysfunction, often manifested as sexual or urinary dysfunction, symmetric sensory impairment and weakness, orthostatic hypotension, diarrhea, and/or impotence.

[0154] Further embodiments of the disclosure are methods of treating a human with transthyretin amyloidosis leading to gastrointestinal disorders and administering to the human a therapeutically effective amount of a compound or composition a described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of diarrhea, constipation, nausea, vomiting, and related kidney and liver disorders.

[0155] Further disclosed is a method for reducing or preventing transthyretin amyloidosis comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing transthyretin amyloidosis.

[0156] Further disclosed is a method for reducing or preventing a cardiac disease comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing a cardiac disease. Further disclosed is a method for reducing or preventing a neuropathic disease comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing a neuropathic disease. Further disclosed is a method for reducing or preventing a gastrointestinal disease

comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing a gastrointestinal disease.

[0157] Further disclosed is a method for ameliorating a symptom of transthyretin amyloidosis, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 4, thereby ameliorating a symptom of transthyretin amyloidosis in the human.

[0158] Further disclosed is a method for reducing the rate of progression of a symptom associated with transthyretin amyloidosis, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 4, thereby reducing the rate of progression a symptom of transthyretin amyloidosis in the human.

[0159] Further disclosed is a method for reversing degeneration indicated by a symptom associated with a transthyretin amyloidosis, administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 4, thereby reversing degeneration indicated by a symptom of transthyretin amyloid disease in the human.

[0160] Further disclosed is a method for ameliorating a symptom of transthyretin amyloidosis, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 80, thereby ameliorating a symptom of transthyretin amyloidosis in the human.

[0161] Further embodiments of the disclosure are methods of treating a human with transthyretin amyloidosis, administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 80, thereby treating transthyretin amyloidosis in a human.

[0162] In certain embodiments, the symptom is a physical, cognitive, psychiatric, or peripheral symptom. In certain embodiments, the symptom is a physical symptom selected from the group consisting of restlessness, lack of coordination, nystagmus, spastic paraparesis, lack of muscle coordination, impaired vision, insomnia, unusual sensations, myoclonus, blindness, loss of speech, Carpal tunnel syndrome, seizures, subarachnoid hemorrhages, stroke and bleeding in the brain, hydrocephalus, ataxia, and spastic paralysis, coma, sensory neuropathy, parathesia, hypesthesia, motor neuropathy, autonomic neuropathy, orthostatic hypotension, cyclic constipation, cyclic diarrhea, nausea, vomiting, reduced sweating, impotence, delayed gastric emptying, urinary retention, urinary incontinence, progressive cardiopathy, fatigue, shortness of breath, weight loss, lack of appetite, numbness, tingling, weakness, enlarged tongue, nephrotic syndrome, congestive heart failure, dyspnea on exertion, peripheral edema, arrhythmias, palpitations, light-headedness, syncope, postural hypotension, peripheral nerve problems, sensory motor impairment, lower limb neuropathy, upper limb neuropathy, hyperalgesia, altered temperature sensation, lower extremity weakness, cachexia, peripheral edema, hepatomegaly, purpura, diastolic dysfunction, premature ventricular contractions, cranial neuropathy, diminished deep tendon reflexes, amyloid deposits in the corpus vitreum, vitreous opacity, dry eyes, glaucoma, scalloped appearance in the pupils, swelling of the feet due to water retention. In certain embodiments, the symptom is a cognitive symptom selected from the group consisting of impaired memory, impaired judgment, and thinking, impaired planning, impaired flexibility, impaired abstract thinking, impaired rule acquisition, impaired initiation of appropriate actions, impaired inhibition of inappropriate actions, impaired short-term memory, impaired long-term memory, paranoia, disorientation, confusion, hallucination and dementia. In certain embodiments, the symptom is a psychiatric symptom selected from the group consisting of dementia; anxiety, depression, blunted affect, egocentrisms, aggression, compulsive behavior, irritability, personality changes, including, impaired memory, judgment, and thinking and suicidal ideation.

[0163] In certain embodiments the symptom is at least one of at least one physical symptom, at least one cognitive symptom, at least one psychiatric symptom, and at least one peripheral symptom.

[0164] In certain embodiments the physical symptom is selected from the group consisting of restlessness, lack of coordination, unintentionally initiated motions, unintentionally uncompleted motions, unsteady gait, chorea, rigidity, writhing motions, abnormal posturing, instability, abnormal facial expressions, difficulty chewing, difficulty swallowing, difficulty speaking, seizure, and sleep disturbances.

[0165] In certain embodiments the cognitive symptom is selected from the group consisting of impaired memory, impaired judgment, and thinking, impaired planning, impaired flexibility, impaired abstract thinking, impaired rule acquisition, impaired initiation of appropriate actions, impaired inhibition of inappropriate actions, impaired short-term memory, impaired long-term memory, paranoia, disorientation, confusion, hallucination and dementia.

[0166] In certain embodiments the psychiatric symptom is selected from the group consisting of dementia; anxiety, depression, blunted affect, egocentrisms, aggression, compulsive behavior, irritability, personality changes, including, impaired memory, judgment, and thinking and suicidal ideation.

[0167] In certain embodiments the peripheral symptom is selected from the group consisting of reduced brain mass, muscle atrophy, cardiac failure, impaired glucose tolerance, weight loss, osteoporosis, and testicular atrophy.

[0168] Also provided are methods and compounds for the preparation of a medicament for the treatment, prevention,

or amelioration of a central nervous system related disease.

[0169] Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, ameliorating, or preventing a transthyretin amyloidosis.

[0170] Certain embodiments provide a compound as described herein for use in treating, preventing, or ameliorating transthyretin amyloidosis 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.

[0171] Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating transthyretin amyloidosis 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.

[0172] Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating transthyretin amyloidosis as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

[0173] Certain embodiments provide a kit for treating, preventing, or ameliorating transthyretin amyloidosis as described herein wherein the kit comprises:

(i) a compound as described herein; and alternatively
(ii) an additional agent or therapy as described herein.

[0174] A kit as described herein may further include instructions for using the kit to treat, prevent, or ameliorate transthyretin amyloidosis as described herein by combination therapy as described herein.

Antisense Compounds

[0175] Oligomeric compounds include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be "antisense" to a target nucleic acid, meaning that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

[0176] 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.

[0177] In certain embodiments, an antisense compound targeted to a transthyretin nucleic acid is 12 to 30 nucleotides in length. In other words, antisense compounds are from 12 to 30 linked nucleobases. In other embodiments, the antisense compound comprises a modified oligonucleotide consisting of 8 to 80, 12 to 50, 12 to 30, 15 to 30, 18 to 24, 18 to 21, 19 to 22, or 20 linked nucleobases. In certain such embodiments, the antisense compound comprises a modified oligonucleotide consisting of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked nucleobases in length, or a range defined by any two of the above values.

[0178] In certain embodiments, the antisense compound comprises a shortened or truncated modified oligonucleotide. The shortened or truncated modified oligonucleotide can have a single nucleoside deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated oligonucleotide may have two nucleosides deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end. Alternatively, the deleted nucleosides may be dispersed throughout the modified oligonucleotide, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

[0179] When a single additional nucleoside is present in a lengthened oligonucleotide, the additional nucleoside may be located at the 5' or 3' end of the oligonucleotide. When two or more additional nucleosides are present, the added nucleosides may be adjacent to each other, for example, in an oligonucleotide having two nucleosides added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the oligonucleotide. Alternatively, the added nucleoside may be dispersed throughout the antisense compound, for example, in an oligonucleotide having one nucleoside added to the 5' end and one subunit added to the 3' end.

[0180] It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly,

target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

[0181] 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.*

[0182] Maher and Dolnick (Nuc. Acid. Res. 16:3341-3358,1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Antisense Compound Motifs

[0183] In certain embodiments, antisense compounds targeted to a transthyretin nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced the inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

[0184] 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.

[0185] Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides or linked nucleosides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides or linked nucleosides that are chemically distinct from the nucleotides or linked nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE, and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH2)nO-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 include, but are not limited to, for example 5-10-5,4-8-4,4-12-3,4-12-4,3-14-3,2-13-5, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1, 2-8-2, 6-8-6 or 5-8-5.

[0186] In certain embodiments, the antisense compound as a "wingmer" motif, having a wing-gap or gap-wing configuration, i.e. an X-Y or Y-Z configuration as described above for the gapmer configuration. Thus, wingmer configurations include, but are not limited to, for example 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, or 5-13.

[0187] In certain embodiments, antisense compounds targeted to a transthyretin nucleic acid possess a 5-10-5 gapmer motif.

[0188] In certain embodiments, antisense compounds targeted to a transthyretin nucleic acid possess a 6-8-6 gapmer motif.

[0189] In certain embodiments, antisense compounds targeted to a transthyretin nucleic acid possess a 5-8-5 gapmer motif.

[0190] In certain embodiments, an antisense compound targeted to a transthyretin nucleic acid has a gap-widened motif.

[0191] In certain embodiments, a gap-widened antisense oligonucleotide targeted to a transthyretin nucleic acid has a gap segment of ten 2'-deoxyribonucleotides positioned immediately adjacent to and between wing segments 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.

[0192] In certain embodiments, a gap-widened antisense oligonucleotide targeted to a transthyretin nucleic acid has a gap segment of eight 2'-deoxyribonucleotides positioned immediately adjacent to and between wing segments 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.

[0193] In certain embodiments, a gap-widened antisense oligonucleotide targeted to a transthyretin nucleic acid has a gap segment of eight 2'-deoxyribonucleotides positioned immediately adjacent to and between wing segments of six chemically modified nucleosides. In certain embodiments, the chemical modification comprises a 2'-sugar modification. In another embodiment, the chemical modification comprises a 2'-MOE sugar modification.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

[0194] In certain embodiments, the transthyretin nucleic acid is any of the sequences set forth in GENBANK Accession No. NM_000371.2, first deposited with GENBANK® on February 13th, 2008 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_010966.10 truncated from nucleotides 2009236 to 2017289, first deposited with GENBANK® on August 1st, 2002 (incorporated herein as SEQ ID NO: 2); exons 1-4 extracted from the rhesus monkey genomic sequence GENBANK Accession No. NW_001105671.1, based on similarity to human exons; and GENBANK Accession No. NW_001105671.1 truncated from nucleotides 628000 to 638000 (incorporated herein as SEQ ID NO: 4), first deposited with GENBANK® on March 28th, 2006.

[0195] It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) or ISIS NO indicate a combination of nucleobase sequence and motif.

[0196] In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for transthyretin can be obtained by accession number from sequence databases such as NCBI. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the target region.

[0197] Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels. In certain embodiments, the desired effect is reduction of levels of protein encoded by the target nucleic acid or a phenotypic change associated with the target nucleic acid.

[0198] A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain embodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

[0199] Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifically exclude a certain structurally defined region such as the start codon or stop codon.

[0200] The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

[0201] 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 transthyretin mRNA levels are indicative of inhibition of transthyretin expression. Reductions in levels of a transthyretin protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of transthyretin expression. For example, increase in brain size to normal, improvement in motor coordination, decrease in continual muscular spasms (dystonia), decrease in irritability and/or anxiety, improvement of memory, or an increase in energy, among other phenotypic changes that may be assayed. Other phenotypic indications, e.g., symptoms associated with transthyretin amyloidosis, may also be assessed as described below.

Hybridization

[0202] In some embodiments, hybridization occurs between an antisense compound disclosed herein and a transthyretin 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.

[0203] 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.

[0204] 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 disclosed herein are specifically hybridizable with a transthyretin nucleic acid.

Complementarity

[0205] 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 transthyretin nucleic acid).

[0206] An antisense compound may hybridize over one or more segments of a transthyretin nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

[0207] In certain embodiments, the antisense compounds disclosed 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 transthyretin 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.

[0208] 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 disclosure. 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).

[0209] In certain embodiments, the antisense compounds disclosed herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, antisense compound may be fully complementary to a transthyretin 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.

[0210] 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.

[0211] 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 transthyretin nucleic acid, or specified portion thereof.

[0212] 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 transthyretin nucleic acid, or specified portion thereof.

[0213] The antisense compounds disclosed herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

[0214] The antisense compounds disclosed herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds disclosed herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

[0215] In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

Modifications

[0216] A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

[0217] Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

[0218] Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

[0219] The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

[0220] Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

[0221] In certain embodiments, antisense compounds targeted to a transthyretin nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

[0222] Antisense compounds of the invention can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substitutent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or $C(R_1)(R_2)$ (R, R_1 and R_2 are each independently H, C_1-C_{12} alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

[0223] Examples of nucleosides having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (R or *S*), 4'-S, 2'-F, 2'-OCH_3, 2'-OCH_2CH_3, 2'-OCH_2CH_2F and 2'-$O(CH_2)_2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH_2)_2SCH_3$, $O(CH_2)_2$-O-$N(R_m)(R_n)$, O-CH_2-C(=O)-$N(R_m)(R_n)$, and O-CH_2-C(=O)-$N(R_1)$-$(CH_2)_2$-$N(R_m)(R_n)$, where each R_1, R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

[0224] As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' and 4'-$CH(CH_2OCH_3)$-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH_2-$N(OCH_3)$-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH_2-O-$N(CH_3)$-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-$C(H)(CH_3)$-2' (see Chattopadhyaya et al., J. Org. Chem., 2009, 74, 118-134); and 4'-CH_2-$C(=CH_2)$-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

[0225] 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).

[0226] 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,

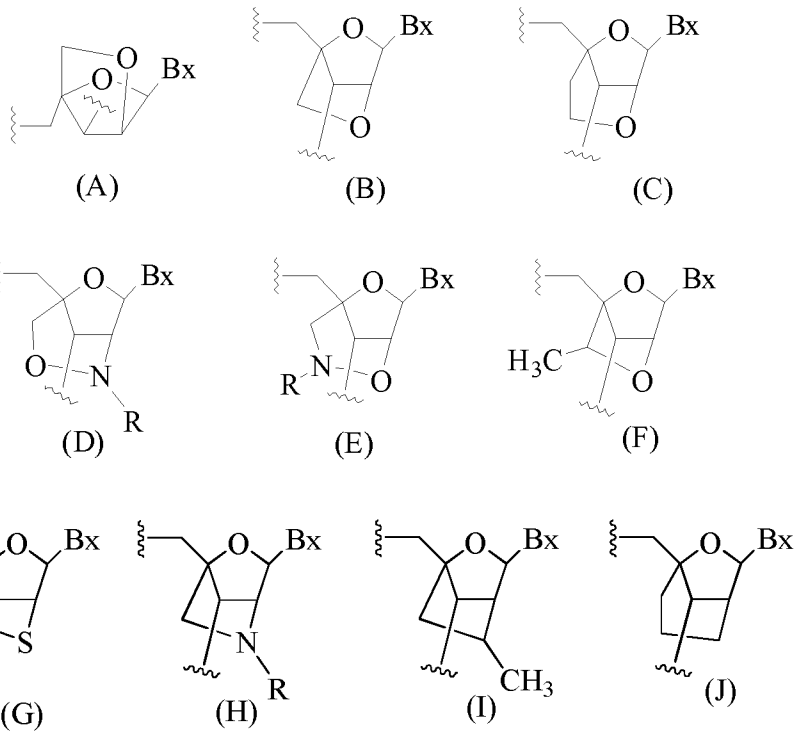
heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl (S(=O)-J_1); and

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

[0227] In certain embodiments, the bridge of a bicyclic sugar moiety is -$[C(R_a)(R_b)]_n$-, -$[C(R_a)(R_b)]_n$-O-, -$C(R_aR_b)$-N(R)-O- or -$C(R_aR_b)$-O-N(R)-. In certain embodiments, the bridge is 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R)-2' and 4'-CH_2-N(R)-O-2'- wherein each R is, independently, H, a protecting group or C_1-C_{12} alkyl.

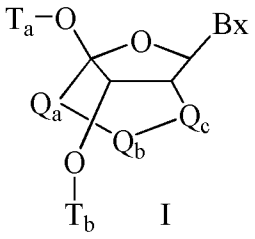
[0228] In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-$(CH_2$-O-2') BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden et al., Nucleic Acids Research, 2003, 21, 6365-6372).

[0229] In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-methyleneoxy (4'-CH_2-O-2') BNA , (B) β-D-methyleneoxy (4'-CH_2-O-2') BNA , (C) ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA , (D) aminooxy (4'-CH_2-O-N(R)-2') BNA, (E) oxyamino (4'-CH_2-N(R)-0-2') BNA, and (F) methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA, (G) methylene-thio (4'-CH_2-S-2') BNA, (H) methylene-amino (4'-CH_2-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH_2-$CH(CH_3)$-2') BNA, and (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA as depicted below.



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

[0230] In certain embodiments, bicyclic nucleosides are provided having Formula I:

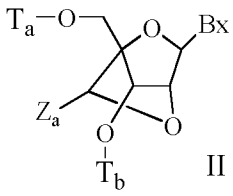


I

wherein:

Bx is a heterocyclic base moiety;
$-Q_a-Q_b-Q_c-$ is $-CH_2-N(R_c)-CH_2-$, $-C(=O)-N(R_c)-CH_2-$, $-CH_2-O-N(R_c)-$, $-CH_2-N(R_c)-O-$ or $-N(R_c)-O-CH_2$;
R_c is C_1-C_{12} alkyl or an amino protecting group; and
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium.

[0231] In certain embodiments, bicyclic nucleosides are provided having Formula II:



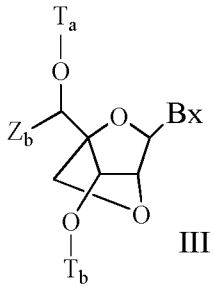
II

wherein:

Bx is a heterocyclic base moiety;
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;
Z_a is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio.

[0232] 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.

[0233] In certain embodiments, bicyclic nucleosides are provided having Formula III:

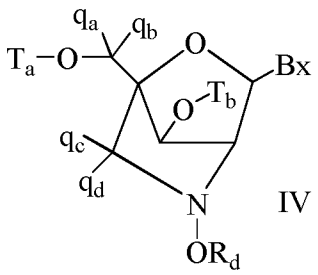


III

wherein:

Bx is a heterocyclic base moiety;
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;
Z_b is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl or substituted acyl (C(=O)-).

[0234] In certain embodiments, bicyclic nucleosides are provided having Formula IV:

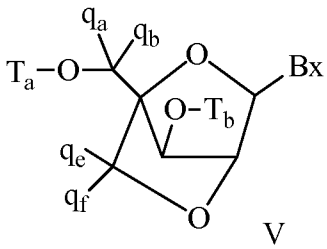


IV

wherein:

Bx is a heterocyclic base moiety;
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;
R_d is C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;
each q_a, q_b, q_c and q_d is, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl, C_1-C_6 alkoxyl, substituted C_1-C_6 alkoxyl, acyl, substituted acyl, C_1-C_6 aminoalkyl or substituted C_1-C_6 aminoalkyl;

[0235] In certain embodiments, bicyclic nucleosides are provided having Formula V:



V

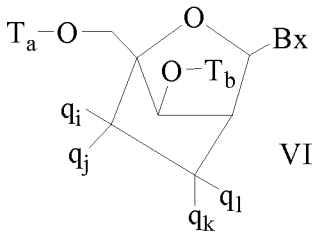
wherein:

Bx is a heterocyclic base moiety;
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;
q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)\text{-}NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;
or q_e and q_f together are $=C(q_g)(q_h)$;
q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl or substituted C_1-C_{12} alkyl.

[0236] The synthesis and preparation of the methyleneoxy (4'-CH_2-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

[0237] Analogs of methyleneoxy (4'-CH_2-O-2') BNA and 2'-thio-BNAs, have also been prepared (Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel et al., WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel comformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

[0238] In certain embodiments, bicyclic nucleosides are provided having Formula VI:



VI

wherein:

Bx is a heterocyclic base moiety;

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

each q_i, q_j, q_k and q_l is, independently, H, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxyl, substituted C_1-C_{12} alkoxyl, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$; and

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

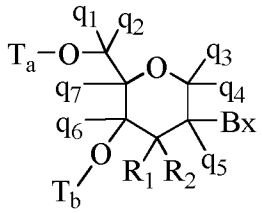
[0239] One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH_2-2' have been described (Freier et al., Nucleic Acids Research, 1997, 25(22), 4429-4443 and Albaek et al., J. Org. Chem., 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava et al., J. Am. Chem. Soc., 2007,129(26), 8362-8379).

[0240] 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.

[0241] As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

[0242] As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C_1-C_{12} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, F, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain (Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- O-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, Helv. Chim. Acta, 1995, 78, 486-504; Altmann et al., Chimia, 1996, 50, 168-176; Altmann et al., Biochem. Soc. Trans., 1996,24, 630-637; and Altmann et al., Nucleosides Nucleotides, 1997,16, 917-926).

[0243] As used herein, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see Leumann, Bioorg. Med. Chem., 2002, 10, 841-854), fluoro HNA (F-HNA) or those compounds having Formula VII:



VII

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

Bx is a heterocyclic base moiety;

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

q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl; and each of R_1 and R_2 is selected from hydrogen, hydroxyl, halogen, subsitituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$ and CN, wherein X is O, S or NJ_1 and each J_1, J_2 and J_3 is, independently, H or C_1-C_6 alkyl.

[0244] In certain embodiments, the modified THP nucleosides of Formula VII are provided wherein q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is other than H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is methyl. In certain embodiments, THP nucleosides of Formula VII are provided wherein one of R_1 and R_2 is fluoro. In certain embodiments, R_1 is fluoro and R_2 is H; R_1 is methoxy and R_2 is H, and R_1 is H and R_2 is methoxyethoxy.

[0245] As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring; and nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, $-OCF_3$, O-$(CH_2)_2$-O-CH_3, 2'-$O(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-$N(R_m)(R_n)$, or O-CH_2-C(=O)-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. 2'-modifed nucleosides may further comprise other modifications, for example at other positions of the sugar and/or at the nucleobase.

[0246] As used herein, "2'-F" refers to a nucleoside comprising a sugar comprising a fluoro group at the 2' position.

[0247] As used herein, "2'-OMe" or "2'-OCH_3" or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an $-OCH_3$ group at the 2' position of the sugar ring.

[0248] As used herein, "MOE" or "2'-MOE" or "2'-$OCH_2CH_2OCH_3$" or "2'-O-methoxyethyl" each refers to a nucleoside comprising a sugar comprising a $-OCH_2CH_2OCH_3$ group at the 2' position of the sugar ring.

[0249] 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).

[0250] 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.

[0251] Methods for the preparations of modified sugars are well known to those skilled in the art.

[0252] 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.

[0253] In certain embodiments, antisense compounds comprise one or more nucleosides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleosides are arranged in a gapmer motif In certain embodiments, the modified sugar moiety is a bicyclic nucleoside having a (4'-$CH(CH_3)$-O-2') bridging group. In certain embodiments, the (4'-$CH(CH_3)$-O-2') modified nucleosides are arranged throughout the wings of a gapmer motif.

Modified Nucleobases

[0254] Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally inter-

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

[0255] Additional unmodified nucleobases include 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[0256] Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Nucleobases that are particularly useful for increasing the binding affinity of antisense compounds include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

[0257] In certain embodiments, antisense compounds targeted to a transthyretin nucleic acid comprise one or more modified nucleobases. In certain embodiments, gap-widened antisense oligonucleotides targeted to a transthyretin nucleic acid comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Compositions and Methods for Formulating Pharmaceutical Compositions

[0258] Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substance for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

[0259] Antisense compound targeted to a transthyretin nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a transthyretin 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.

[0260] Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

[0261] 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

[0262] 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.

[0263] 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

[0264] The effects of antisense compounds on the level, activity or expression of transthyretin 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 cells are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HepG2 cells, Hep3B cells, primary hepatocytes, A549 cells, GM04281 fibroblasts and LLC-MK2 cells.

In vitro testing of antisense oligonucleotides

[0265] Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

[0266] In general, cells are treated with antisense oligonucleotides when the cells reach approximately 60-80% confluence in culture.

[0267] 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.

[0268] Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE 2000® (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE 2000® 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.

[0269] Another reagent used to introduce antisense oligonucleotides into cultured cells includes Cytofectin® (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with Cytofectin® in OPTI-MEM® 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a Cytofectin® concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

[0270] Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

[0271] 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.

[0272] 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 LIPOFECTAMINE2000®, Lipofectin or Cytofectin. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

[0273] 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

[0274] Inhibition of levels or expression of a transthyretin nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitaive real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM® 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

[0275] 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.

[0276] Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT and real-time PCR reagents are obtained from Invitrogen (Carlsbad, CA). RT, real-time-PCR reactions are carried out by methods well known to those skilled in the art.

[0277] Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN® (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN® RNA quantification reagent (Invitrogen, Inc. Eugene, OR). Methods of RNA quantification by RIBOGREEN® are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR® 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN® fluorescence.

[0278] Probes and primers are designed to hybridize to a transthyretin nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS® Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

[0279] Antisense inhibition of transthyretin nucleic acids can be assessed by measuring transthyretin protein levels. Protein levels of transthyretin can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of human and rat transthyretin are commercially available.

In vivo testing of antisense compounds

[0280] Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of transthyretin and produce phenotypic changes. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration. Following a period of treatment with antisense oligonucleotides, RNA is isolated from tissue and changes in transthyretin nucleic acid expression are measured. Changes in transthyretin protein levels are also measured.

Certain Compounds

[0281] About two hundred and forty six newly designed antisense compounds of various lengths, motifs and backbone composition were tested for their effect on human transthyretin mRNA *in vitro* in several cell types. The new compounds were compared with about seventy nine previously designed compounds including ISIS NOs. 304267, 304268, 304280, 304284, 304285, 304286, 304287, 304288, 304289, 304290, 304291, 304292, 304293, 304294, 304296, 304297, 304298, 304299, 304300, 304301, 304302, 304303, 304304, 304307, 304308, 304309, 304311, and 304312 which have previously been determined to be some of the most potent antisense compounds *in vitro* (see e.g., U.S. Patent Publication Nos. US2005/0244869 and US2009/0082300). Of the about three hundred and twenty five newly designed and previously designed antisense compounds, about fifteen compounds were selected for further study based on *in vitro* potency. The selected compounds were tested for in vivo potency and tolerability in a transgenic mouse model (see Example 10). Of the fifteen compounds tested, eleven were selected and tested for systemic tolerability (see Example 11) and half-life measurement in liver (see Example 12) in CD1 mice, and also for systemic tolerability (see Example 13) and pharmacokinetic studies of oligonucleotide concentration in liver (see Example 14) in Sprague-Dawley rats. From these studies, seven compounds were tested for dose dependent inhibition and tolerability in transgenic mice (see Example 15). Furthermore, fifteen additional compounds were selected from Table 1 and six additional compounds with various motifs were designed with overlapping sequences to ISIS 420951, which displayed high potency and

tolerability in the above-mentioned assays. These additional compounds were compared with ISIS 420951 for potency and tolerability in transgenic mice (see Example 16). Based on all these studies (Examples 10-16), twenty two compounds were selected and tested for systemic tolerability in CD1 mice (see Example 17). Seven compounds were considered tolerable in the mouse model and further tested for systemic tolerability in Sprague-Dawley rats (see Example 18) and for pharmacokinetic studies of oligonucleotide concentration in the liver and kidney (see Example 19). The seven compounds were also tested for dose-dependent potency in transgenic mice (see Example 20).

[0282] Final evaluation of these studies (Examples 16-20), led to the selection of nine compounds having a nucleobase sequence of a sequence recited in SEQ ID NO: 25, 78, 80, 86, 87, 115, 120, 122 and 124. By virtue of their complementary sequence, the compounds are complementary to the regions 505-524, 507-526, 508-527, 513-532, 515-534, 516-535, 580-599, 585-604, 587-606, or 589-608 of SEQ ID NO: 1. In certain embodiments, the compounds targeting the listed regions, as further described herein, comprise a modified oligonucleotide having some nucleobase portion of the sequence recited in the SEQ ID NOs, as further described herein, In certain embodiments, the compounds targeting the listed regions or having a nucleobase portion of a sequence recited in the listed SEQ ID NOs can be of various length, as further described herein, and can have one of various motifs, as further described herein. In certain embodiments, a compound targeting a region or having a nucleobase portion of a sequence recited in the listed SEQ ID NOs has the specific length and motif as indicated by the ISIS NOs: ISIS 304299, ISIS 420913, ISIS 420915, ISIS 420921, ISIS 420922, ISIS 420950, ISIS 420955, ISIS 420957, or ISIS 420959.

[0283] The nine compounds having a nucleobase sequence of a sequence recited in SEQ ID NO: 25, 78, 80, 86, 87, 115, 120, 122 and 124, were further tested for dose dependent inhibition in primary hepatocytes of cynomolgus monkey (See Example 21). These compounds were also tested for optimal viscosity (Example 22).The half life in the liver of CD1 mice of seven of the compounds having a nucleobase sequence of a sequence recited in SEQ ID NOs: 78, 86, 87. 115, 120 and 124 was also evaluated (Example 23).

[0284] Final evaluation of these studies (Examples 1-23), led to the selection of eight compounds having a nucleobase sequence of a sequence recited in SEQ ID NO: 25, 80, 86, 87, 115, 120, 122 and 124. By virtue of their complementary sequence, the compounds are complementary to the regions 504-523, 505-524, 512-531, 513-532, 577-596, 582-601, 584-603, and 586-605 of SEQ ID NO: 1. In certain embodiments, the compounds targeting the listed regions, as further described herein, comprise a modified oligonucleotide having some nucleobase portion of the sequence recited in the SEQ ID NOs, as further described herein, In certain embodiments, the compounds targeting the listed regions or having a nucleobase portion of a sequence recited in the listed SEQ ID NOs can be of various length, as further described herein, and can have one of various motifs, as further described herein. In certain embodiments, a compound targeting a region or having a nucleobase portion of a sequence recited in the listed SEQ ID NOs has the specific length and motif as indicated by the ISIS NOs: ISIS 304299, ISIS 420915, ISIS 420921, ISIS 420922, ISIS 420950, ISIS 420955, ISIS 420957, or ISIS 420959.

[0285] These eight compounds were tested for efficacy, pharmacokinetic profile and tolerability in cynomolgus monkeys (Example 24). The inhibition studies in these monkeys indicated that treatment with some of these compounds caused high inhibition of TTR mRNA in the liver. Specifically, treatment with ISIS 420950, ISIS 420955 and ISIS 420915 caused 91%, 79% and 78% inhibition, respectively compared to the PBS control. It was noted that ISIS 420915 caused greater inhibition of TTR (78%) mRNA compared to ISIS 304299 (59%), even though the two oligonucleotides differ from each other by a single base-pair shift of their target region on SEQ ID NO: 1. Protein analysis also complemented the RNA analysis data with treatment with ISIS 420915 causing 76% inhibition and treatment with ISIS 304299 causing 47% inhibition of TTR protein compared to the control. RBP4 protein levels, as a protein associated with transthyretin, was also expected to decrease after treatment with the antisense compounds. RBP4 protein levels decreased by 63% after treatment with ISIS 420915. Treatment with ISIS 304299 decreased RBP4 protein levels by 19%. Additionally, ISIS 420915 was more tolerable than ISIS 304299, as indicated in the monkey study (Example 24) Transaminase levels of monkeys treated with ISIS 304299 (ALT 81 IU/L and AST 101 IU/L) were higher than those treated with ISIS 420915 (ALT 68 IU/L and AST 62 IU/L). The complement C3 levels of monkeys treated with ISIS 304299 (96 mg/dL) were lower than that of monkeys treated with ISIS 420915 (104 mg/dL).

[0286] Accordingly, disclosed herein are antisense compounds with any one or more of the improved characteristics. In a certain embodiments, disclosed herein are compounds comprising a modified oligonucleotide as further described herein targeted to or specifically hybridizable with the region of nucleotides of SEQ ID NO: 1.

[0287] Accordingly, disclosed herein are antisense compounds with any one or more of the improved characteristics. In a certain embodiments, disclosed herein are compounds comprising a modified oligonucleotide as further described herein targeted to or specifically hybridizable with the region of nucleotides of SEQ ID NO: 2.

[0288] Accordingly, disclosed herein are antisense compounds with any one or more of the improved characteristics. In a certain embodiments, disclosed herein are compounds comprising a modified oligonucleotide as further described herein targeted to or specifically hybridizable with the region of nucleotides of SEQ ID NO: 4.

[0289] In certain embodiments, the compounds as described herein are efficacious by virtue of having at least one of an *in vitro* IC_{50} of less than 2.9 uM, less than 2.2 uM, less than 2.0 uM, less than 1.5 uM, less than 1.4 uM, less than

1.3 uM, less than 1.0 uM, less than 0.7 uM, less than 0.6 uM, when delivered to a cynomolgous monkey hepatocyte cell line using electroporation as described in Example 67. In certain embodiments, the compounds as described herein are highly tolerable as demonstrated by having at least one of an increase in ALT or AST value of no more than 4 fold, 3 fold, or 2 fold over saline treated animals; or an increase in liver, spleen or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5% or 2%.

Certain Indications

[0290] In certain embodiments, disclosed herein are methods of treating an individual comprising administering one or more pharmaceutical compositions as described herein. In certain embodiments, the individual has central nervous system related disease.

[0291] As shown in the examples below, compounds targeted to transthyretin as described herein have been shown to reduce the severity of physiological symptoms of central nervous system related diseases. In certain of the experiments, the compounds reduced rate of amyloid plaque formation, e.g., the animals continued to experience symptoms, but the symptoms were less severe compared to untreated animals. In other of the experiments, however, the compounds appear to result in regeneration of function over time; e.g., animals treated for a longer period of time experienced less severe symptoms than those administered the compounds for a shorter period of time. The ability of the compounds exemplified below to restore function therefore demonstrates that symptoms of the disease may be reversed by treatment with a compound as described herein.

[0292] Accordingly, disclosed herein are methods for ameliorating a symptom associated with central nervous system related, cardiac, neuropathologic or gastrointestinal disease in a subject in need thereof. In certain embodiments, disclosed is a method for reducing the rate of onset of a symptom associated with central nervous system related, cardiac, neuropathologic or gastrointestinal disease. In certain embodiments, disclosed is a method for reducing the severity of a symptom associated with central nervous system related, cardiac, neuropathologic or gastrointestinal. In such embodiments, the methods comprise administering to an individual in need thereof a therapeutically effective amount of a compound targeted to a Transthyretin nucleic acid.

[0293] Transthyretin amyloidosis is characterized by numerous physical, neurological, psychiatric, and/or peripheral symptoms. Any symptom known to one of skill in the art to be associated with transthyretin amyloidosis can be ameliorated or otherwise modulated as set forth above in the methods described above. In certain embodiments, the symptom is a physical, cognitive, psychiatric, or peripheral symptom. In certain embodiments, the symptom is a physical symptom selected from the group consisting of restlessness, lack of coordination, nystagmus, spastic paraparesis, lack of muscle coordination, impaired vision, insomnia, unusual sensations, myoclonus, blindness, loss of speech, Carpal tunnel syndrome, seizures, subarachnoid hemorrhages, stroke and bleeding in the brain, hydrocephalus, ataxia, and spastic paralysis, coma, sensory neuropathy, parathesia, hypesthesia, motor neuropathy, autonomic neuropathy, orthostatic hypotension, cyclic constipation, cyclic diarrhea, nausea, vomiting, reduced sweating, impotence, delayed gastric emptying, urinary retention, urinary incontinence, progressive cardiopathy, fatigue, shortness of breath, weight loss, lack of appetite, numbness, tingling, weakness, enlarged tongue, nephrotic syndrome, congestive heart failure, dyspnea on exertion, peripheral edema, arrhythmias, palpitations, light-headedness, syncope, postural hypotension, peripheral nerve problems, sensory motor impairment, lower limb neuropathy, upper limb neuropathy, hyperalgesia, altered temperature sensation, lower extremity weakness, cachexia, peripheral edema, hepatomegaly, purpura, diastolic dysfunction, premature ventricular contractions, cranial neuropathy, diminished deep tendon reflexes, amyloid deposits in the corpus vitreum, vitreous opacity, dry eyes, glaucoma, scalloped appearance in the pupils, swelling of the feet due to water retention. In certain embodiments, the symptom is a cognitive symptom selected from the group consisting of impaired memory, impaired judgment, and thinking, impaired planning, impaired flexibility, impaired abstract thinking, impaired rule acquisition, impaired initiation of appropriate actions, impaired inhibition of inappropriate actions, impaired short-term memory, impaired long-term memory, paranoia, disorientation, confusion, hallucination and dementia. In certain embodiments, the symptom is a psychiatric symptom selected from the group consisting of dementia; anxiety, depression, blunted affect, egocentrisms, aggression, compulsive behavior, irritability, personality changes, including, impaired memory, judgment, and thinking and suicidal ideation.

[0294] In certain embodiments, the symptom is restlessness. In certain embodiments, the symptom is lack of coordination. In certain embodiments, the symptom is nystagmus. In certain embodiments, the symptom is spastic paraparesis. In certain embodiments, the symptom is lack of muscle coordination. In certain embodiments, the symptom is impaired vision. In certain embodiments, the symptom is insomnia. In certain embodiments, the symptom is unusual sensations. In certain embodiments, the symptom is myoclonus. In certain embodiments, the symptom is blindness. In certain embodiments, the symptom is loss of speech. In certain embodiments, the symptom is Carpal tunnel syndrome. In certain embodiments, the symptom is seizures. In certain embodiments, the symptom is subarachnoid hemorrhages. In certain embodiments, the symptom is stroke. In certain embodiments, the symptom is bleeding in the brain. In certain embodiments, the symptom is hydrocephalus. In certain embodiments, the symptom is ataxia. In certain embodiments,

the symptom is spastic paralysis. In certain embodiments, the symptom is coma. In certain embodiments, the symptom is sensory neuropathy. In certain embodiments, the symptom is parathesia. In certain embodiments, the symptom is hypesthesia. In certain embodiments, the symptom is motor neuropathy. In certain embodiments, the symptom is autonomic neuropathy. In certain embodiments, the symptom is orthostatic hypotension. In certain embodiments, the symptom is cyclic constipation. In certain embodiments, the symptom is cyclic diarrhea. In certain embodiments, the symptom is nausea. In certain embodiments, the symptom is vomiting. In certain embodiments, the symptom is reduced sweating. In certain embodiments, the symptom is impotence. In certain embodiments, the symptom is delayed gastric emptying. In certain embodiments, the symptom is urinary retention. In certain embodiments, the symptom is urinary incontinence. In certain embodiments, the symptom is progressive cardiopathy. In certain embodiments, the symptom is fatigue. In certain embodiments, the symptom is shortness of breath. In certain embodiments, the symptom is weight loss. In certain embodiments, the symptom is numbness. In certain embodiments, the symptom is tingling. In certain embodiments, the symptom is weakness. In certain embodiments, the symptom is enlarged tongue. In certain embodiments, the symptom is nephrotic syndrome. In certain embodiments, the symptom is congestive heart failure. In certain embodiments, the symptom is dyspnea on exertion. In certain embodiments, the symptom is peripheral edema. In certain embodiments, the symptom is arrhythmias. In certain embodiments, the symptom is palpitations. In certain embodiments, the symptom is light-headedness. In certain embodiments, the symptom is syncope. In certain embodiments, the symptom is postural hypotension. In certain embodiments, the symptom is peripheral nerve problems. In certain embodiments, the symptom is sensory motor impairment. In certain embodiments, the symptom is lower limb neuropathy. In certain embodiments, the symptom is upper limb neuropathy. In certain embodiments, the symptom is hyperalgesia. In certain embodiments, the symptom is altered temperature sensation. In certain embodiments, the symptom is lower extremity weakness. In certain embodiments, the symptom is cachexia. In certain embodiments, the symptom is edema. In certain embodiments, the symptom is hepatomegaly. In certain embodiments, the symptom is purpura. In certain embodiments, the symptom is diastolic dysfunction. In certain embodiments, the symptom is premature ventricular contractions. In certain embodiments, the symptom is cranial neuropathy. In certain embodiments, the symptom is diminished deep tendon reflexes. In certain embodiments, the symptom is amyloid deposits in the corpus vitreum. In certain embodiments, the symptom is vitreous opacity. In certain embodiments, the symptom is dry eyes. In certain embodiments, the symptom is glaucoma. In certain embodiments, the symptom is scalloped appearance in the pupils. In certain embodiments, the symptom is swelling of the feet due to water retention.

[0295] In certain embodiments, the symptom is impaired memory. In certain embodiments, the symptom is impaired judgment, and thinking. In certain embodiments, the symptom is impaired planning. In certain embodiments, the symptom is impaired flexibility. In certain embodiments, the symptom is impaired abstract thinking. In certain embodiments, the symptom is impaired rule acquisition. In certain embodiments, the symptom is impaired initiation of appropriate actions. In certain embodiments, the symptom is impaired inhibition of inappropriate actions. In certain embodiments, the symptom is impaired short-term memory. In certain embodiments, the symptom is impaired long-term memory. In certain embodiments, the symptom is paranoia. In certain embodiments, the symptom is disorientation. In certain embodiments, the symptom is confusion. In certain embodiments, the symptom is hallucination. In certain embodiments, the symptom is dementia.

[0296] In certain embodiments, the symptom is dementia. In certain embodiments, the symptom is anxiety. In certain embodiments, the symptom is depression. In certain embodiments, the symptom is blunted affect. In certain embodiments, the symptom is egocentrisms. In certain embodiments, the symptom is aggression. In certain embodiments, the symptom is compulsive behavior. In certain embodiments, the symptom is irritability. In certain embodiments, the symptom is personality changes. In certain embodiments, the symptom is suicidal ideation.

[0297] In certain embodiments, disclosed are methods of treating an individual comprising administering one or more pharmaceutical compositions as described herein. In certain embodiments, the individual has central nervous system related disease.

[0298] In certain embodiments, administration of an antisense compound targeted to a transthyretin nucleic acid results in reduction of transthyretin expression by at least about 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.

[0299] In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to transthyretin are used for the preparation of a medicament for treating a patient suffering or susceptible to central nervous system related disease.

[0300] In certain embodiments, the methods described herein include administering a compound comprising a modified oligonucleotide having a contiguous nucleobases portion as described herein of a sequence recited in SEQ ID NO: 25, 78, 80, 86, 87, 115, 120, 122 and 124.

Administration

[0301] In certain embodiments, the compounds and compositions as described herein may be administered in a

number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. The compounds and compositions as described herein can be delivered in a manner to target a particular tissue, such as the liver or brain.

[0302] In certain embodiments, the compounds and compositions as described herein are administered parenterally. "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. intracerebral administration, intrathecal administration, intraventricular administration, ventricular administration, intracerebroventricular administration, cerebral intraventricular administration or cerebral ventricular administration. Administration can be continuous, or chronic, or short or intermittent.

[0303] In certain embodiments, parenteral administration is by infusion. Infusion can be chronic or continuous or short or intermittent. In certain embodiments, infused pharmaceutical agents are delivered with a pump. In certain embodiments, parenteral administration is by injection.

[0304] In certain embodiments, parenteral administration is subcutaneous.

[0305] In further embodiments, the formulation for administration is the compounds described herein and saline.

[0306] In certain embodiments, compounds and compositions are delivered to the CNS. In certain embodiments, compounds and compositions are delivered to the cerebrospinal fluid. In certain embodiments, compounds and compositions are administered to the brain parenchyma. In certain embodiments, compounds and compositions are delivered to an animal into multiple regions of the central nervous system (e.g., into multiple regions of the brain, and/or into the spinal cord) by intrathecal administration, or intracerebroventricular administration. Broad distribution of compounds and compositions, described herein, within the central nervous system may be achieved with intraparenchymal administration, intrathecal administration, or intracerebroventricular administration.

[0307] In certain embodiments, the present invention includes pharmaceutical compositions that can be delivered by injection directly into the brain. The injection can be by stereotactic injection into a particular region of the brain (e.g., the substantia nigra, choroid plexus, cortex, hippocampus, striatum, choroid plexus or globus pallidus). The compound can also be delivered into diffuse regions of the brain (e.g., diffuse delivery to the cortex of the brain).

[0308] In certain embodiments, parenteral administration is by injection. The injection may be delivered with a syringe or a pump. In certain embodiments, the injection is a bolus injection. In certain embodiments, the injection is administered directly to a tissue, such as striatum, caudate, cortex, hippocampus and cerebellum.

[0309] In certain embodiments, delivery of a compound or composition described herein can affect the pharmacokinetic profile of the compound or composition. In certain embodiments, injection of a compound or composition described herein, to a targeted tissue improves the pharmacokinetic profile of the compound or composition as compared to infusion of the compound or composition. In a certain embodiment, the injection of a compound or composition improves potency compared to broad diffusion, requiring less of the compound or composition to achieve similar pharmacology. In certain embodiments, similar pharmacology refers to the amount of time that a target mRNA and/or target protein is downregulated (e.g. duration of action). In certain embodiments, methods of specifically localizing a pharmaceutical agent, such as by bolus injection, decreases median effective concentration (EC50) by a factor of about 50 (e.g. 50 fold less concentration in tissue is required to achieve the same or similar pharmacodynamic effect). In certain embodiments, methods of specifically localizing a pharmaceutical agent, such as by bolus injection, decreases median effective concentration (EC50) by a factor of 20, 25, 30, 35, 40, 45 or 50. In certain embodiments the pharmaceutical agent in an antisense compound as further described herein. In certain embodiments, the targeted tissue is brain tissue. In certain embodiments the targeted tissue is striatal tissue. In certain embodiments, decreasing EC50 is desirable because it reduces the dose required to achieve a pharmacological result in a patient in need thereof.

[0310] The half-life of MOE gapmer oligonucleotides in CD1 mice liver tissue is about 21 days (see Examples 12).

[0311] In certain embodiments, an antisense oligonucleotide is delivered by injection or infusion once every month, every two months, every 90 days, every 3 months, every 6 months, twice a year or once a year.

Certain Combination Therapies

[0312] In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease, disorder, or condition as the one or more pharmaceutical compositions 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 as described herein. In certain embodiments, one or more pharmaceutical compositions 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 are co-administered with another pharmaceutical agent to

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

[0313] In certain embodiments, one or more pharmaceutical compositions and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions and one or more other pharmaceutical agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions and one or more other pharmaceutical agents are prepared separately.

[0314] In certain embodiments, the second compound is administered prior to administration of a pharmaceutical composition of the present invention. In certain embodiments, the second compound is administered following administration of a pharmaceutical composition of the present invention. In certain embodiments, the second compound is administered at the same time as a pharmaceutical composition of the present invention. In certain embodiments, the dose of a co-administered second compound is the same as the dose that would be administered if the second compound was administered alone. In certain embodiments, the dose of a co-administered second compound is lower than the dose that would be administered if the second compound was administered alone. In certain embodiments, the dose of a co-administered second compound is greater than the dose that would be administered if the second compound was administered alone.

[0315] In certain embodiments, the co-administration of a second compound enhances the effect of a first compound, such that co-administration of the compounds results in an effect that is greater than the effect of administering the first compound alone. In certain embodiments, the co-administration results in effects that are additive of the effects of the compounds when administered alone. In certain embodiments, the co-administration results in 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.

[0316] In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include antipsychotic agents, such as, *e.g.*, haloperidol, chlorpromazine, clozapine, quetapine, and olanzapine; antidepressant agents, such as, *e.g.*, fluoxetine, sertraline hydrochloride, venlafaxine and nortriptyline; tranquilizing agents such as, *e.g.*, benzodiazepines, clonazepam, paroxetine, venlafaxin, and beta-blockers; mood-stabilizing agents such as, *e.g.*, lithium, valproate, lamotrigine, and carbamazepine; paralytic agents such as, *e.g.*, Botulinum toxin; and/or other experimental agents including, but not limited to, tetrabenazine (Xenazine), creatine, conezyme Q10, trehalose, docosahexanoic acids, ACR16, ethyl-EPA, atomoxetine, citalopram, dimebon, memantine, sodium phenylbutyrate, ramelteon, ursodiol, zyprexa, xenasine, tiapride, riluzole, amantadine, [123I]MNI-420, atomoxetine, tetrabenazine, digoxin, detromethorphan, warfarin, alprozam, ketoconazole, omeprazole, and minocycline.

[0317] In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include analgesics, such as, paracetamol (acetaminophen); non-steroidal anti-inflammatory drugs (NSAIDs), such as, salicylates; narcotic drugs, such as, morphine, and synthetic drugs with narcotic properties such as tramadol.

In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include muscle relaxants, such as, benzodiapines and methocarbamol.

Formulations

[0318] The compounds of the invention may also be admixed, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S.: 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756.

[0319] The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof.

[0320] The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Patent 6,287,860. Sodium salts have been shown to be suitable forms of oligonucleotide drugs.

[0321] The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number

of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intracerebral administration, intrathecal administration, intraventricular administration, ventricular administration, intracerebroventricular administration, cerebral intraventricular administration or cerebral ventricular administration.

[0322] Administration intraventricularly, is preferred to target transthyretin expression in the choroid plexus. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

[0323] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0324] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0325] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

[0326] Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Patent 6,287,860.

[0327] Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

[0328] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Liposomes and their uses are further described in U.S. Patent 6,287,860.

[0329] In another embodiment of the invention, formulations of the present invention include saline formulations. In certain embodiment of the invention, a formulation consists of the compounds described herein and saline. In certain embodiments, a formulation consists essentially of the compounds described herein and saline. In certain embodiments, the saline is pharmaceutically acceptable grade saline. In certain embodiments, the saline is buffered saline. In certain embodiments, the saline is phosphate buffered saline (PBS).

[0330] In certain embodiments, a formulation excludes liposomes. In certain embodiments, the formulation excludes sterically stabilized liposomes. In certain embodiments, a formulation excludes phospholipids. In certain embodiments, the formulation consists essentially of the compounds described herein and saline and excludes liposomes.

[0331] The pharmaceutical formulations and compositions of the present invention may also include surfactants. Surfactants and their uses are further described in U.S. Patent 6,287,860.

[0332] In one embodiment, the present invention employs various penetration enhancers to affect the efficient delivery of nucleic acids, particularly oligonucleotides. Penetration enhancers and their uses are further described in U.S. Patent 6,287,860.

[0333] One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

[0334] Preferred formulations for topical administration include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidyl DOPE ethanolamine,

dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA).

[0335] Compositions and formulations for parenteral administration, including intravenous, intraarterial, subcutaneous, intraperitoneal, intramuscular injection or infusion, or intracranial may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0336] Certain embodiments of the invention disclose pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the compounds of the invention, such chemotherapeutic agents may be used individually (*e.g.*, 5-FU and oligonucleotide), sequentially (*e.g.*, 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (*e.g.*, 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

[0337] In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Alternatively, compositions of the invention may contain two or more antisense compounds targeted to different regions of the same nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

Dosing

[0338] The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or at desired intervals. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, once or more daily.

[0339] While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

EXAMPLES

Non-limiting disclosure

[0340] 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.

Example 1: Antisense inhibition of human transthyretin in HepG2 cells

[0341] Antisense oligonucleotides were designed targeting a transthyretin nucleic acid and were tested for their effects on transthyretin mRNA in vitro. Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin reagent with 50 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated

from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS1396 (forward sequence CCCTGCTGAGCCCCTACTC, designated herein as SEQ ID NO: 5; reverse sequence TCCCTCATTCCTTGGGATTG, designated herein as SEQ ID NO: 6; probe sequence ATTCCACCACGGCTGTCGTCAX , designated herein as SEQ ID NO: 7). Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells.

[0342] The chimeric antisense oligonucleotides in Tables 1 and 2 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleotides each. Each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Human Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the human gene sequence. "Human Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted human gene sequence. Each gapmer listed in Table 1 is targeted to human transthyretin mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_000371.2). Certain gapmers were also designed which targeted intronic sequences or intron-exon junctions of the human transthyretin genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_010966.10 truncated from nucleotides 2009236 to 2017289) and are listed in Table 2.

[0343] The human oligonucleotides of Tables 1 and 2 are also cross-reactive with rhesus monkey gene sequences. 'Mismatches' indicate the number of nucleobases by which the human oligonucleotide is mismatched with a rhesus monkey gene sequence. 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. The human oligonucleotides in Table 1 were compared to exons 1-4 extracted from the rhesus monkey genomic sequence GENBANK Accession No. NW_001105671.1, based on similarity to human exons. The human oligonucleotides in Table 2 were compared to the rhesus monkey genomic sequence, designated herein as SEQ ID NO: 4 (GENBANK Accession No. NW_001105671.1 truncated from nucleotides 628000 to 638000). "Rhesus monkey Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the rhesus monkey gene sequence. "Rhesus monkey Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted rhesus monkey gene sequence.

Table 1

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
304267	217	236	coding	ACTGGTTTTCC CAGAGGCAA	53	217	236	0	8
304268	222	241	coding	GACTCACTGGT TTTCCCAGA	16	222	241	0	9
304280	353	372	coding	TGAATACCAC CTCTGCATGC	51	353	372	0	10
304284	425	444	coding	CCGTGGTGGA ATAGGAGTAG	82	425	444	0	11
304285	427	446	coding	AGCCGTGGTG GAATAGGAGT	89	427	446	0	12
304286	431	450	coding	CGACAGCCGT GGTGGAATAG	63	431	450	0	13
304287	438	457	coding	TTGGTGACGA CAGCCGTGGT	88	438	457	0	14
304288	440	459	coding	GATTGGTGAC GACAGCCGTG	82	440	459	0	15
304289	442	461	coding	GGGATTGGTG ACGACAGCCG	78	442	461	0	16
304290	443	462	coding	TGGGATTGGT GACGACAGCC	85	443	462	0	17
304291	449	468	coding -stop codon	ATTCCTTGGGA TTGGTGACG	52	449	468	0	18
304292	450	469	coding -stop codon	CATTCCTTGGG ATTGGTGAC	34	450	469	0	19
304293	451	470	coding -stop codon	TCATTCCTTGG GATTGGTGA	29	451	470	0	20

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(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
304294	460	479	coding -stop codon-3'UTR	AGAAGTCCCT CATTCCTTGG	32	460	479	0	21
304296	481	500	3'-UTR	GTCCTTCAGGT CCACTGGAG	84	478	497	2	22
304297	489	508	3'-UTR	CATCCCTCGTC CTTCAGGTC	0	486	505	1	23
304298	501	520	3'-UTR	TACATGAAAT CCCATCCCTC	26	498	517	0	24
304299	507	526	3'-UTR	CTTGGTTACAT GAAATCCCA	85	504	523	0	25
304300	513	532	3'-UTR	AATACTCTTGG TTACATGAA	49	510	529	0	26
304301	526	545	3'UTR	TTAGTAAAAA TGGAATACTC	0	523	542	0	27
304302	532	551	3'UTR	ACTGCTTTAGT AAAAATGGA	42	529	548	0	28
304303	539	558	3'UTR	TGAAAACACT GCTTTAGTAA	41	536	555	0	29
304304	546	565	3'UTR	TATGAGGTGA AAACACTGCT	49	543	562	0	30
304307	564	583	3'UTR	TGGACTTCTAA CATAGCATA	73	561	580	2	31
304308	572	591	3'UTR	TCTCTGCCTGG ACTTCTAAC	55	569	588	1	32
304309	578	597	3'-UTR	TTATTGTCTCT GCCTGGACT	77	575	594	0	33

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
304311	597	616	3'-UTR	TGCCTTTCACA GGAATGTTT	80	594	613	0	34
304312	598	617	3'-UTR	GTGCCTTTCAC AGGAATGTT	71	595	614	0	35
420871	36	55	coding	CAGAGGAGGA GCAGACGATG	48	36	55	0	36
420872	120	139	coding	TCTAGAACTTT GACCATCAG	55	120	139	0	37
420873	212	231	coding	TTTTCCCAGAG GCAAATGGC	54	212	231	0	38
420874	226	245	coding	TCCAGACTCAC TGGTTTTCC	63	226	245	0	39
420875	271	290	coding	TATCCCTTCTA CAAATTCCT	40	271	290	0	40
420876	285	304	coding	ATTTCCACTTT GTATATCCC	42	285	304	0	41
420877	293	312	coding	TGGTGTCTATT TCCACTTTG	76	293	312	0	42
420878	303	322	coding	CAGTAAGATTT GGTGTCTAT	80	303	322	0	43
420879	307	326	coding	CTTCCAGTAAG ATTTGGTGT	73	307	326	0	44
420880	347	366	coding	CCACCTCTGCA TGCTCATGG	76	347	366	0	45
420881	355	374	coding	TGTGAATACC ACCTCTGCAT	58	355	374	0	46

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
420882	357	376	coding	GCTGTGAATA CCACCTCTGC	69	357	376	0	47
420883	362	381	coding	CGTTGGCTGTG AATACCACC	64	362	381	0	48
420884	428	447	coding	CAGCCGTGGT GGAATAGGAG	93	428	447	0	49
420885	430	449	coding	GACAGCCGTG GTGGAATAGG	93	430	449	0	50
420886	432	451	coding	ACGACAGCCG TGGTGGAATA	92	432	451	0	51
420887	433	452	coding	GACGACAGCC GTGGTGGAAT	93	433	452	0	52
420888	434	453	coding	TGACGACAGC CGTGGTGGAA	95	434	453	0	53
420889	435	454	coding	GTGACGACAG CCGTGGTGGA	93	435	454	0	54
420890	436	455	coding	GGTGACGACA GCCGTGGTGG	97	436	455	0	55
420891	437	456	coding	TGGTGACGAC AGCCGTGGTG	97	437	456	0	56
420892	439	458	coding	ATTGGTGACG ACAGCCGTGG	93	439	458	0	57
420893	441	460	coding	GGATTGGTGA CGACAGCCGT	96	441	460	0	58
420894	444	463	coding	TTGGGATTGGT GACGACAGC	88	444	463	0	59

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
420895	445	464	coding	CTTGGGATTGG TGACGACAG	95	445	464	0	60
420896	446	465	coding	CCTTGGGATTG GTGACGACA	95	446	465	0	61
420897	447	466	coding	TCCTTGGGATT GGTGACGAC	94	447	466	0	62
420898	448	467	coding -stop codon	TTCCTTGGGAT TGGTGACGA	86	448	467	0	63
420899	452	471	coding -stop codon-3'UTR	CTCATTCCTTG GGATTGGTG	94	452	471	0	64
420900	453	472	coding -stop codon-3'UTR	CCTCATTCCTT GGGATTGGT	92	453	472	0	65
420901	454	473	coding -stop codon-3'UTR	CCCTCATTCCT TGGGATTGG	93	454	473	0	66
420902	455	474	coding -stop codon-3'UTR	TCCCTCATTCC TTGGGATTG	75	455	474	0	67
420903	456	475	coding -stop codon-3'UTR	GTCCCTCATTC CTTGGGATT	57	456	475	0	68
420904	457	476	coding -stop codon-3'UTR	AGTCCCTCATT CCTTGGGAT	62	457	476	0	69
420905	458	477	coding -stop codon-3'UTR	AAGTCCCTCAT TCCTTGGGA	58	458	477	0	70
420906	459	478	coding -stop codon-3'UTR	GAAGTCCCTC ATTCCTTGGG	79	459	478	0	71
420907	461	480	coding -stop codon-3'UTR	GAGAAGTCCC TCATTCCTTG	59	461	480	0	72

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
420908	462	481	coding -stop codon-3'UTR	GGAGAAGTCC CTCATTCCTT	75	462	481	0	73
420909	500	519	3'UTR	ACATGAAATC CCATCCCTCG	82	497	516	0	74
420910	502	521	3'UTR	TTACATGAAAT CCCATCCCT	74	499	518	0	75
420911	503	522	3'UTR	GTTACATGAA ATCCCATCCC	81	500	519	0	76
420912	504	523	3'UTR	GGTTACATGA AATCCCATCC	92	501	520	0	77
420913	505	524	3'UTR	TGGTTACATGA AATCCCATC	95	502	521	0	78
420914	506	525	3'UTR	TTGGTTACATG AAATCCCAT	93	503	522	0	79
420915	508	527	3'UTR	TCTTGGTTACA TGAAATCCC	92	505	524	0	80
420916	509	528	3'UTR	CTCTTGGTTAC ATGAAATCC	88	506	525	0	81
420917	510	529	3'UTR	ACTCTTGGTTA CATGAAATC	92	507	526	0	82
420918	511	530	3'UTR	TACTCTTGGTT ACATGAAAT	88	508	527	0	83
420919	512	531	3'UTR	ATACTCTTGGT TACATGAAA	89	509	528	0	84
420920	514	533	3'UTR	GAATACTCTTG GTTACATGA	87	511	530	0	85

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
420921	515	534	3'UTR	GGAATACTCTT GGTTACATG	92	512	531	0	86
420922	516	535	3'UTR	TGGAATACTCT TGGTTACAT	95	513	532	0	87
420923	517	536	3'UTR	ATGGAATACT CTTGGTTACA	90	514	533	0	88
420924	518	537	3'UTR	AATGGAATAC TCTTGGTTAC	75	515	534	0	89
420925	519	538	3'UTR	AAATGGAATA CTCTTGGTTA	87	516	535	0	90
420926	520	539	3'UTR	AAAATGGAAT ACTCTTGGTT	88	517	536	0	91
420927	521	540	3'UTR	AAAAATGGAA TACTCTTGGT	50	518	537	0	92
420928	522	541	3'UTR	TAAAAATGGA ATACTCTTGG	26	519	538	0	93
420929	523	542	3'UTR	GTAAAAATGG AATACTCTTG	56	520	539	0	94
420930	524	543	3'UTR	AGTAAAAATG GAATACTCTT	18	521	540	0	95
420931	525	544	3'UTR	TAGTAAAAAT GGAATACTCT	12	522	541	0	96
420932	527	546	3'UTR	TTTAGTAAAA ATGGAATACT	1	524	543	0	97
420933	528	547	3'UTR	CTTTAGTAAAA ATGGAATAC	0	525	544	0	98

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
420934	529	548	3'UTR	GCTTTAGTAAA AATGGAATA	6	526	545	0	99
420935	530	549	3'UTR	TGCTTTAGTAA AATGGAAT	0	527	546	0	100
420936	531	550	3'UTR	CTGCTTTAGTA AAAATGGAA	40	528	547	0	101
420937	533	552	3'UTR	CACTGCTTTAG TAAAAATGG	47	530	549	0	102
420938	534	553	3'UTR	ACACTGCTTTA GTAAAAATG	30	531	550	0	103
420939	535	554	3'UTR	AACACTGCTTT AGTAAAAAT	0	532	551	0	104
420940	536	555	3'UTR	AAACACTGCTT TAGTAAAAA	0	533	552	0	105
420941	537	556	3'UTR	AAAACACTGC TTTAGTAAAA	0	534	553	0	106
420942	538	557	3'UTR	GAAAACACTG CTTTAGTAAA	0	535	554	0	107
420943	540	559	3'UTR	GTGAAAACAC TGCTTTAGTA	14	537	556	0	108
420944	541	560	3'UTR	GGTGAAAACA CTGCTTTAGT	43	538	557	0	109
420945	542	561	3'UTR	AGGTGAAAAC ACTGCTTTAG	41	539	558	0	110

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
420946	543	562	3'UTR	GAGGTGAAAA CACTGCTTTA	20	540	559	0	111
420947	544	563	3'UTR	TGAGGTGAAA ACACTGCTTT	69	541	560	0	112
420948	545	564	3'UTR	ATGAGGTGAA AACACTGCTT	63	542	561	0	113
420949	579	598	3'UTR	TTTATTGTCTC TGCCTGGAC	84	576	595	0	114
420950	580	599	3'UTR	TTTTATTGTCT CTGCCTGGA	69	577	596	0	115
420951	581	600	3'UTR	GTTTTATTGTC TCTGCCTGG	87	578	597	0	116
420952	582	601	3'UTR	TGTTTTATTGT CTCTGCCTG	67	579	598	0	117
420953	583	602	3'UTR	ATGTTTTATTG TCTCTGCCT	51	580	599	0	118
420954	584	603	3'UTR	AATGTTTTATT GTCTCTGCC	60	581	600	0	119
420955	585	604	3'UTR	GAATGTTTTAT TGTCTCTGC	65	582	601	0	120
420956	586	605	3'UTR	GGAATGTTTTA TTGTCTCTG	67	583	602	0	121
420957	587	606	3'UTR	AGGAATGTTTT ATTGTCTCT	68	584	603	0	122
420958	588	607	3'UTR	CAGGAATGTTT TATTGTCTC	45	585	604	0	123

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1 and SEQ ID NO: 4								
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	SEQ ID NO
420959	589	608	3'UTR	ACAGGAATGT TTTATTGTCT	28	586	605	0	124

Table 2

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 2 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	ID NO
420960	606	625	exonl-intron1	GATGTCACAG AAACACTCAC	13	1755	1774	0	125
420961	665	684	intron 1	GCAAAGCTGG AAGGAGTCAC	7	1814	1833	0	126
420962	748	767	intron 1	GAACTTCATTC TTTTTGAAG	0	1897	1916	0	127
420963	882	901	intron 1	AGCTTCCTTAA TATCATATC	0	2031	2050	0	128
420964	966	985	intron 1	TATAGGGCCA GAATATAATC	10	2115	2134	0	129
420965	1010	1029	intron 1	ACTAAGCCTTT TAAAGATTA	17	2159	2178	0	130
420966	1208	1227	intron 1	TGGAATTACT GAAAAGATGT	35	2356	2375	0	131
420967	1289	1308	intron 1	ACCAGGGATG TGTATAATGA	43	2437	2456	0	132
420968	1364	1383	intron 1	TCCCTACTCAG TATAACACA	0	2512	2531	0	133
420969	1472	1491	intron 1	GATCAGAGTG AAAGGATTTA	0	2620	2639	0	134
420970	1687	1706	intron 2	GGGAAGATAA AACCAAGTCC	46	2826	2845	0	135
420971	1739	1758	intron 2	TAAATTCTTTA GCAGATGAT	0	2878	2897	0	136
420972	1842	1861	intron 2	AATGATGCTC AGGTTCCTGG	23	2980	2999	0	137

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 2 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	ID NO
420973	2051	2070	intron 2	TTGGTGTTACC CAGGGACAC	0	3187	3206	0	138
420974	2207	2226	intron 2	AAAGTGTTCA TTAGGCAAAA	29	3344	3363	0	139
420975	2655	2674	intron 2	GGCATTTTATA TAAACATAA	0	3798	3817	0	140
420976	2733	2752	intron 2	AAGAACATTG GAATATTTTT	0	3876	3895	0	141
420977	2874	2893	intron 2	GTTGGAAATT GCTTCCCATT	9	4017	4036	0	142
420978	3015	3034	intron 2	AGTGGAAAAC CTAAAGTAGG	0	4156	4175	0	143
420979	3618	3637	intron 2	TTCCCCTCAAC TAAGTCAGA	0	4795	4814	0	144
420980	3735	3754	intron2 -exon 3	CCTATAAGGT GTGAAAGTCT	0	4930	4949	0	145
420981	4096	4115	intron 3	TGTAAGTTCA AGTCATGTTA	0	5291	5310	0	146
420982	4306	4325	intron 3	GTGTTGCCAA GAATCACTTG	0	5502	5521	0	147
420983	4404	4423	intron 3	AAAACACTTA TAATTGTGTC	0	5600	5619	0	148
420984	4518	4537	intron 3	CTTTGACAAGT TATTTGACT	0	5714	5733	0	149
420985	4880	4899	intron 3	ATCCATGACT AAGCCAGAGA	0	6073	6092	0	150

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 2 and SEQ ID NO: 4									
ISIS NO	Human Start Site	Human Stop Site	Region	Sequence	% inhibition	Rhesus monkey start site	Rhesus monkey stop site	Mismatches	ID NO
470986	5185	5204	intron 3	ATGGTTCCCAT CAGGCTGAG	0	6379	6398	0	151
420987	5542	5561	intron 3	GCATTTATCAG AAGAAGCTG	0	6732	6751	0	152
420988	6030	6049	intron 3	TTGACCTTCAG CCCACTTGA	0	7226	7245	0	153
420989	6133	6152	intron 3	AGGAAGTGAG AATCACCTAA	0	7641	7660	0	154
420990	6320	6339	intron 3	AGAAGACAGT AAAGATGTGT	0	7828	7847	0	155
420991	6457	6476	intron 3	AAATTGTGGA TCAAAATGCT	0	7966	7985	0	156
420992	6736	6755	intron 3	AACCAGACTT GAATTATTGT	0	8246	8265	0	157
420993	6811	6830	intron 3	AGTGGCTGCC AACCACAGAC	0	8321	8340	0	158
420994	7106	7125	intron 3	GGAAGTCCAG TGCCAACTTA	0	8615	8634	0	159
420995	7162	7181	intron 3	ATCCATTTCCA CCAGAGCCC	0	8670	8689	0	160

[0344] Due to the short length of the human transthyretin mRNA, a second primer probe set was designed away from the first primer probe set, RTS1396, to avoid amplicon oligonucleotides. The antisense oligonucleotides were also tested for their effects on transthyretin mRNA in vitro using new human primer probe set RTS3029 (forward sequence CTTGCTGGACTGGTATTTGTGTCT, designated herein as SEQ ID NO: 161, reverse sequence AGAACTTTGACCATCAGAGGACACT, designated herein as SEQ ID NO: 162; probe sequence CCCTACGGGCACCGGTGAATCCX, designated herein as SEQ ID NO: 163). Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin reagent with 50 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. The results are presented in Table 3 as percent inhibition of the PBS control cell set.

Table 3

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap with primer probe set RTS3029		
ISIS NO	Region	% inhibition
304267	coding	13
304268	coding	10
304280	coding	23
304284	coding	10
304285	coding	34
304286	coding	0
304287	coding	34
304288	coding	45
304289	coding	3
304290	coding	16
304291	coding-stop codon	4
304292	coding-stop codon	10
304293	coding-stop codon	14
304294	stop codon-3' UTR	30
304296	exon 4	78
304297	exon 4	29
304298	exon 4	19
304299	exon 4	85
304300	exon 4	52
304301	exon 4	15
304302	exon 4	45
304303	exon 4	51
304304	exon 4	62
304307	exon 4	76
304308	exon 4	63
304309	exon 4	75
304311	exon 4	81
304312	exon 4	68

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap with primer probe set RTS3029		
420871	coding	0
420872	coding	5
420873	coding	19
420874	coding	0
420875	coding	6
420876	coding	20
420877	coding	28
420878	coding	37
420879	coding	34
420880	coding	36
420881	coding	10
420882	coding	27
420883	coding	13
420884	coding	28
420885	coding	4
420886	coding	21
420887	coding	39
420888	coding	37
420889	coding	9
420890	coding	27
420891	coding	39
420892	coding	43
420893	coding	39
420894	coding	0
420895	coding	0
420896	coding	24
420897	coding	31
420898	coding-	0
420899	stop codon-3'UTR	41
420900	stop codon-3'UTR	26
420901	stop codon-3'UTR	28
420902	stop codon-3'UTR	20
420903	stop codon-3'UTR	20
420904	stop codon-3'UTR	22
420905	stop codon-3'UTR	32
420906	stop codon-3'UTR	13
420907	-stop codon-3'UTR	0

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap with primer probe set RTS3029		
420908	stop codon-3'UTR	45
420909	3'UTR	41
420910	3'UTR	14
420911	3'UTR	45
420912	3'UTR	62
420913	3'UTR	81
420914	3'UTR	68
420915	3'UTR	71
420916	3'UTR	54
420917	3'UTR	50
420918	3'UTR	43
420919	3'UTR	65
420920	3'UTR	61
420921	3'UTR	65
420922	3'UTR	68
420923	3'UTR	62
420924	3'UTR	9
420925	3'UTR	17
420926	3'UTR	47
420927	3'UTR	57
420928	3'UTR	51
420929	3'UTR	46
420930	3'UTR	39
420931	3'UTR	14
420932	3'UTR	6
420933	3'UTR	1
420934	3'UTR	48
420935	3'UTR	13
420936	3'UTR	62
420937	3'UTR	65
420938	3'UTR	48
420939	3'UTR	7
420940	3'UTR	3
420941	3'UTR	31
420942	3'UTR	0
420943	3'UTR	40
420944	3'UTR	78

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap with primer probe set RTS3029		
420945	3'UTR	58
420946	3'UTR	52
420947	3'UTR	71
420948	3'UTR	73
420949	3'UTR	88
420950	3'UTR	82
420951	3'UTR	90
420952	3'UTR	82
420953	3'UTR	71
420954	3'UTR	67
420955	3'UTR	73
420956	3'UTR	65
420957	3'UTR	74
420958	3'UTR	69
420959	3'UTR	63
420960	exon1-intron1	14
420961	intron 1	16
420962	intron 1	0
420963	intron 1	0
420964	intron 1	14
420965	intron 1	23
420966	intron 1	25
420967	intron 1	12
420968	intron 1	0
420969	intron 1	0
420970	intron 2	25
420971	intron 2	0
420972	intron 2	25
420973	intron 2	7
420974	intron 2	28
420975	intron 2	9
420976	intron 2	21
420977	intron 2	14
420978	intron 2	37
420979	intron 2	37
420980	intron2-exon 3	16
420981	intron 3	0

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap with primer probe set RTS3029		
420982	intron 3	28
420983	intron 3	0
420984	intron 3	0
420985	intron 3	0
420986	intron 3	7
420987	intron 3	0
420988	intron 3	0
420989	intron 3	0
420990	intron 3	6
420991	intron 3	15
420992	intron 3	0
420993	intron 3	0
420994	intron 3	0
420995	intron 3	10

[0345] Based on the inhibition results using the new primer probe set RTS3029, antisense oligonucleotides exhibiting 50% or more inhibition of transthyretin mRNA were selected for further studies.

Example 2: Antisense inhibition of human transthyretin in HepG2 cells by oligonucleotides designed by microwalk

[0346] Additional gapmers were designed based on the gapmers presented in Table 3 that demonstrated an inhibition of at least 50%. These gapmers were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of the original gapmers from Table 3. Gapmers were also created with various motifs, e.g. 5-10-5 MOE, 3-14-3 MOE, 2-13-5 MOE, and 4-11-5 MOE motifs. These gapmers were tested *in vitro.* Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin reagent with 50 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. The human primer probe set RTS3029 was used to measure transthyretin mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. The results are presented in Table 4.

[0347] The chimeric antisense oligonucleotides in Table 4 were designed as 5-10-5 MOE, 3-14-3 MOE, 2-13-5 MOE or 4-11-5 MOE gapmers. The gapmers designated with an asterisk (*) in Table 4 are the original gapmers from which gapmers, ISIS 425650-425763, were designed via microwalk. The 5-10-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleotides each. The 3-14-3 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of fourteen 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three nucleotides each. The 2-13-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of thirteen 2'-deoxynucleotides and is flanked on the 5' and the 3' directions with wings comprising two and five nucleotides respectively. The 4-11-5 gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of eleven 2'-deoxynucleotides and is flanked on the 5' and the 3' directions with wings comprising four and five nucleotides respectively. For each of the motifs (5-10-5, 3-14-3, 2-113-5, and 4-11-5), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 4 is targeted to the target region spanning nucleobases 481-619 of SEQ ID NO: 1 (GENBANK Accession No. NM_000371.2).

[0348] As shown in Table 4, several of the gapmers exhibited at least 50% inhibition, including ISIS numbers: 304296, 425655, 425695, 425735, 425649, 425656, 425696, 425736, 420912, 425657, 425697, 425737, 420913, 425658, 425698, 425738, 420914, 425659, 425699, 425739, 304299, 425660, 425700, 425740,420915,420916,425662,425702,420919,425703,420920,425664,425704,425742,420921, 425665, 425705, 425743, 420922, 425666, 425706, 420923, 420937, 420944, 425669, 425709, 425746, 425710, 425711, 425747, 420948, 425712, 425748, 425673, 425713, 425749, 425651, 425675, 425715, 425751, 304309, 425676, 425716, 425752, 420949, 425677, 425717, 425753, 420950, 425678, 425718, 425754, 420951, 425679, 425719, 425755, 420952, 425680, 425720, 425756, 420953, 425681, 425721, 425757, 420954, 425722, 425758, 420955, 425759, 425724, 425760, 425762, 304310, 425729, 425764, 425653, 425690, 425730, 425765, 304311, 425691, 425731; 425766, 304312, 425692, 425732, 425767, 425654, 425693, 425733, 425768, 304313, 425734, and 425769.

[0349] Several of the gapmers exhibited at least 60% inhibition, including ISIS numbers: 304296, 425655, 425695, 425735, 425649, 425656, 425696, 425736, 420912, 425657, 425697, 425737, 420913, 425658, 425698, 425738, 420914, 425659, 425739, 304299, 425740, 420915, 425702, 420919, 420920, 425742, 420921, 425665, 425705, 425706, 420923, 425746, 42571 I, 425747, 420948, 425712, 425748, 425651, 425715, 425751, 304309, 425716, 425752, 425677, 425717, 425753, 420950, 425718, 425754, 420951, 425679, 425719, 425755, 420952, 425680, 425720, 420953, 425681, 425721, 425757, 420954, 425722, 425758, 420955, 425724, 425760, 425764, 425653, 425690, 425730, 425765, 304311, 425691, 425731, 425766, 304312, 425692, 425732, 425767, 425654, 425693, 425733, 304313, and 425769.

[0350] Several of the gapmers exhibited at least 70% inhibition, including ISIS numbers: 304296, 425655, 425695, 425735, 425649, 425656, 425696, 425736, 420912, 425657, 425737, 420913, 425738, 420914, 425659, 304299, 420915, 420920, 425742, 425712, 425748, 425716, 425754, 420951, 425679, 425719, 425755, 425680, 425721, 425757, 425760, 425653, 425690, 425730, 425765, 304311, 425691, 425731, 425766, 304312, 425767, 425693, and 304313.

[0351] Several of the gapmers exhibited at least 80% inhibition, including ISIS numbers: 304296, 425655, 425695, 425736, 420913, 425659, 304299, 420915, 425716, 425754, 425719, 425757, 425765, and 425767.

[0352] Several of the gapmers exhibited at least 85% inhibition, including ISIS numbers: 420913, 425716, 425754, and 425719.

[0353] One gapmer, ISIS 425719, exhibited 90% inhibition.

Table 4

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000371.2)						
OligoID	Start Site	Stop Site	Sequence	Motif	% inhibition	SEQ ID NO
*304296	481	500	GTCCTTCAGGTCCACTGGAG	5-10-5	83	22
425655	481	500	GTCCTTCAGGTCCACTGGAG	3-14-3	80	22
425695	481	500	GTCCTTCAGGTCCACTGGAG	2-13-5	80	22
425735	481	500	GTCCTTCAGGTCCACTGGAG	4-11-5	72	22
425649	482	501	CGTCCTTCAGGTCCACTGGA	5-10-5	75	170
425656	482	501	CGTCCTTCAGGTCCACTGGA	3-14-3	78	170
425696	482	501	CGTCCTTCAGGTCCACTGGA	2-13-5	74	170
425736	482	501	CGTCCTTCAGGTCCACTGGA	4-11-5	83	170
*420912	504	523	GGTTACATGAAATCCCATCC	5-10-5	73	77
425657	504	523	GGTTACATGAAATCCCATCC	3-14-3	76	77
425697	504	523	GGTTACATGAAATCCCATCC	2-13-5	69	77
425737	504	523	GGTTACATGAAATCCCATCC	4-11-5	78	77
*420913	505	524	TGGTTACATGAAATCCCATC	5-10-5	89	78
425658	505	524	TGGTTACATGAAATCCCATC	3-14-3	69	78
425698	505	524	TGGTTACATGAAATCCCATC	2-13-5	61	78
425738	505	524	TGGTTACATGAAATCCCATC	4-11-5	78	78

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000371.2)						
OligoID	Start Site	Stop Site	Sequence	Motif	% inhibition	SEQ ID NO
*420914	506	525	TTGGTTACATGAAATCCCAT	5-10-5	70	79
425659	506	525	TTGGTTACATGAAATCCCAT	3-14-3	83	79
425699	506	525	TTGGTTACATGAAATCCCAT	2-13-5	56	79
425739	506	525	TTGGTTACATGAAATCCCAT	4-11-5	69	79
*304299	507	526	CTTGGTTACATGAAATCCCA	5-10-5	83	25
425660	507	526	CTTGGTTACATGAAATCCCA	3-14-3	59	25
425700	507	526	CTTGGTTACATGAAATCCCA	2-13-5	52	25
425740	507	526	CTTGGTTACATGAAATCCCA	4-11-5	69	25
*420915	508	527	TCTTGGTTACATGAAATCCC	5-10-5	81	80
425661	508	527	TCTTGGTTACATGAAATCCC	3-14-3	48	80
425701	508	527	TCTTGGTTACATGAAATCCC	2-13-5	41	80
425741	508	527	TCTTGGTTACATGAAATCCC	4-11-5	37	80
*420916	509	528	CTCTTGGTTACATGAAATCC	5-10-5	52	81
425662	509	528	CTCTTGGTTACATGAAATCC	3-14-3	57	81
425702	509	528	CTCTTGGTTACATGAAATCC	2-13-5	63	81
*420919	512	531	ATACTCTTGGTTACATGAAA	5-10-5	69	84
425663	512	531	ATACTCTTGGTTACATGAAA	3-14-3	46	84
425703	512	531	ATACTCTTGGTTACATGAAA	2-13-5	52	84
*420920	514	533	GAATACTCTTGGTTACATGA	5-10-5	71	85
425664	514	533	GAATACTCTTGGTTACATGA	3-14-3	57	85
425704	514	533	GAATACTCTTGGTTACATGA	2-13-5	58	85
425742	514	533	GAATACTCTTGGTTACATGA	4-11-5	71	85
*420921	515	534	GGAATACTCTTGGTTACATG	5-10-5	68	86
425665	515	534	GGAATACTCTTGGTTACATG	3-14-3	65	86
425705	515	534	GGAATACTCTTGGTTACATG	2-13-5	60	86
425743	515	534	GGAATACTCTTGGTTACATG	4-11-5	56	86
*420922	516	535	TGGAATACTCTTGGTTACAT	5-10-5	54	87
425666	516	535	TGGAATACTCTTGGTTACAT	3-14-3	56	87
425706	516	535	TGGAATACTCTTGGTTACAT	2-13-5	64	87
425744	516	535	TGGAATACTCTTGGTTACAT	4-11-5	39	87
*420923	517	536	ATGGAATACTCTTGGTTACA	5-10-5	62	88
425667	517	536	ATGGAATACTCTTGGTTACA	3-14-3	44	88
425707	517	536	ATGGAATACTCTTGGTTACA	2-13-5	30	88
*420937	533	552	CACTGCTTTAGTAAAAATGG	5-10-5	59	102
425668	533	552	CACTGCTTTAGTAAAAATGG	3-14-3	37	102

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000371.2)						
OligoID	Start Site	Stop Site	Sequence	Motif	% inhibition	SEQ ID NO
425708	533	552	CACTGCTTTAGTAAAAATGG	2-13-5	32	102
425745	533	552	CACTGCTTTAGTAAAAATGG	4-11-5	43	102
*420944	541	560	GGTGAAAACACTGCTTTAGT	5-10-5	52	109
425669	541	560	GGTGAAAACACTGCTTTAGT	3-14-3	54	109
425709	541	560	GGTGAAAACACTGCTTTAGT	2-13-5	54	109
425746	541	560	GGTGAAAACACTGCTTTAGT	4-11-5	60	109
*420945	542	561	AGGTGAAAACACTGCTTTAG	5-10-5	38	110
425670	542	561	AGGTGAAAACACTGCTTTAG	3-14-3	38	110
425710	542	561	AGGTGAAAACACTGCTTTAG	2-13-5	52	110
*420947	544	563	TGAGGTGAAAACACTGCTTT	5-10-5	34	112
425671	544	563	TGAGGTGAAAACACTGCTTT	3-14-3	27	112
425711	544	563	TGAGGTGAAAACACTGCTTT	2-13-5	68	112
425747	544	563	TGAGGTGAAAACACTGCTTT	4-11-5	61	112
*420948	545	564	ATGAGGTGAAAACACTGCTT	5-10-5	66	113
425672	545	564	ATGAGGTGAAAACACTGCTT	3-14-3	47	113
425712	545	564	ATGAGGTGAAAACACTGCTT	2-13-5	70	113
425748	545	564	ATGAGGTGAAAACACTGCTT	4-11-5	71	113
*304304	546	565	TATGAGGTGAAAACACTGCT	5-10-5	46	30
425673	546	565	TATGAGGTGAAAACACTGCT	3-14-3	51	30
425713	546	565	TATGAGGTGAAAACACTGCT	2-13-5	50	30
425749	546	565	TATGAGGTGAAAACACTGCT	4-11-5	58	30
425650	547	566	ATATGAGGTGAAAACACTGC	5-10-5	28	171
425674	547	566	ATATGAGGTGAAAACACTGC	3-14-3	40	171
425714	547	566	ATATGAGGTGAAAACACTGC	2-13-5	44	171
425750	547	566	ATATGAGGTGAAAACACTGC	4-11-5	47	171
425651	577	596	TATTGTCTCTGCCTGGACTT	5-10-5	65	172
425675	577	596	TATTGTCTCTGCCTGGACTT	3-14-3	55	172
425715	577	596	TATTGTCTCTGCCTGGACTT	2-13-5	65	172
425751	577	596	TATTGTCTCTGCCTGGACTT	4-11-5	62	172
*304309	578	597	TTATTGTCTCTGCCTGGACT	5-10-5	66	33
425676	578	597	TTATTGTCTCTGCCTGGACT	3-14-3	59	33
425716	578	597	TTATTGTCTCTGCCTGGACT	2-13-5	87	33
425752	578	597	TTATTGTCTCTGCCTGGACT	4-11-5	67	33
*420949	579	598	TTTATTGTCTCTGCCTGGAC	5-10-5	57	114
425677	579	598	TTTATTGTCTCTGCCTGGAC	3-14-3	67	114

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000371.2)						
OligoID	Start Site	Stop Site	Sequence	Motif	% inhibition	SEQ ID NO
425717	579	598	TTTATTGTCTCTGCCTGGAC	2-13-5	68	114
425753	579	598	TTTATTGTCTCTGCCTGGAC	4-11-5	69	114
*420950	580	599	TTTTATTGTCTCTGCCTGGA	5-10-5	61	115
425678	580	599	TTTTATTGTCTCTGCCTGGA	3-14-3	59	115
425718	580	599	TTTTATTGTCTCTGCCTGGA	2-13-5	69	115
425754	580	599	TTTTATTGTCTCTGCCTGGA	4-11-5	86	115
*420951	581	600	GTTTTATTGTCTCTGCCTGG	5-10-5	78	116
425679	581	600	GTTTTATTGTCTCTGCCTGG	3-14-3	73	116
425719	581	600	GTTTTATTGTCTCTGCCTGG	2-13-5	90	116
425755	581	600	GTTTTATTGTCTCTGCCTGG	4-11-5	73	116
*420952	582	601	TGTTTTATTGTCTCTGCCTG	5-10-5	61	117
425680	582	601	TGTTTTATTGTCTCTGCCTG	3-14-3	77	117
425720	582	601	TGTTTTATTGTCTCTGCCTG	2-13-5	67	117
425756	582	601	TGTTTTATTGTCTCTGCCTG	4-11-5	57	117
*420953	583	602	ATGTTTTATTGTCTCTGCCT	5-10-5	65	118
425681	583	602	ATGTTTTATTGTCTCTGCCT	3-14-3	61	118
425721	583	602	ATGTTTTATTGTCTCTGCCT	2-13-5	77	118
425757	583	602	ATGTTTTATTGTCTCTGCCT	4-11-5	83	118
*420954	584	603	AATGTTTTATTGTCTCTGCC	5-10-5	63	119
425682	584	603	AATGTTTTATTGTCTCTGCC	3-14-3	42	119
425722	584	603	AATGTTTTATTGTCTCTGCC	2-13-5	69	119
425758	584	603	AATGTTTTATTGTCTCTGCC	4-11-5	61	119
*420955	585	604	GAATGTTTTATTGTCTCTGC	5-10-5	65	120
425683	585	604	GAATGTTTTATTGTCTCTGC	3-14-3	30	120
425723	585	604	GAATGTTTTATTGTCTCTGC	2-13-5	44	120
425759	585	604	GAATGTTTTATTGTCTCTGC	4-11-5	50	120
*420956	586	605	GGAATGTTTTATTGTCTCTG	5-10-5	47	121
425684	586	605	GGAATGTTTTATTGTCTCTG	3-14-3	44	121
425724	586	605	GGAATGTTTTATTGTCTCTG	2-13-5	65	121
*420957	587	606	AGGAATGTTTTATTGTCTCT	5-10-5	37	122
425685	587	606	AGGAATGTTTTATTGTCTCT	3-14-3	46	122
425725	587	606	AGGAATGTTTTATTGTCTCT	2-13-5	43	122
425760	587	606	AGGAATGTTTTATTGTCTCT	4-11-5	78	122
*420958	588	607	CAGGAATGTTTTATTGTCTC	5-10-5	41	123
425686	588	607	CAGGAATGTTTTATTGTCTC	3-14-3	6	123

(continued)

Inhibition of human transthyretin mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000371.2)						
OligoID	Start Site	Stop Site	Sequence	Motif	% inhibition	SEQ ID NO
425726	588	607	CAGGAATGTTTTATTGTCTC	2-13-5	41	123
425761	588	607	CAGGAATGTTTTATTGTCTC	4-11-5	39	123
*420959	589	608	ACAGGAATGTTTTATTGTCT	5-10-5	43	124
425687	589	608	ACAGGAATGTTTTATTGTCT	3-14-3	22	124
425727	589	608	ACAGGAATGTTTTATTGTCT	2-13-5	25	124
425762	589	608	ACAGGAATGTTTTATTGTCT	4-11-5	57	124
425652	590	609	CACAGGAATGTTTTATTGTC	5-10-5	23	173
425688	590	609	CACAGGAATGTTTTATTGTC	3-14-3	11	173
425728	590	609	CACAGGAATGTTTTATTGTC	2-13-5	37	173
425763	590	609	CACAGGAATGTTTTATTGTC	4-11-5	38	173
304310	595	614	CCTTTCACAGGAATGTTTTA	5-10-5	57	174
425689	595	614	CCTTTCACAGGAATGTTTTA	3-14-3	38	174
425729	595	614	CCTTTCACAGGAATGTTTTA	2-13-5	58	174
425764	595	614	CCTTTCACAGGAATGTTTTA	4-11-5	60	174
425653	596	615	GCCTTTCACAGGAATGTTTT	5-10-5	79	175
425690	596	615	GCCTTTCACAGGAATGTTTT	3-14-3	73	175
425730	596	615	GCCTTTCACAGGAATGTTTT	2-13-5	76	175
425765	596	615	GCCTTTCACAGGAATGTTTT	4-11-5	83	175
*304311	597	616	TGCCTTTCACAGGAATGTTT	5-10-5	71	34
425691	597	616	TGCCTTTCACAGGAATGTTT	3-14-3	74	34
425731	597	616	TGCCTTTCACAGGAATGTTT	2-13-5	73	34
425766	597	616	TGCCTTTCACAGGAATGTTT	4-11-5	79	34
*304312	598	617	GTGCCTTTCACAGGAATGTT	5-10-5	71	35
425692	598	617	GTGCCTTTCACAGGAATGTT	3-14-3	69	35
425732	598	617	GTGCCTTTCACAGGAATGTT	2-13-5	67	35
425767	598	617	GTGCCTTTCACAGGAATGTT	4-11-5	83	35
425654	599	618	AGTGCCTTTCACAGGAATGT	5-10-5	64	176
425693	599	618	AGTGCCTTTCACAGGAATGT	3-14-3	79	176
425733	599	618	AGTGCCTTTCACAGGAATGT	2-13-5	68	176
425768	599	618	AGTGCCTTTCACAGGAATGT	4-11-5	50	176
304313	600	619	AAGTGCCTTTCACAGGAATG	5-10-5	73	177
425694	600	619	AAGTGCCTTTCACAGGAATG	3-14-3	45	177
425734	600	619	AAGTGCCTTTCACAGGAATG	2-13-5	55	177
425769	600	619	AAGTGCCTTTCACAGGAATG	4-11-5	62	177

Example 3: Dose-dependent antisense inhibition of human transthyretin in HepG2 cells

[0354] Gapmers from Example 2 exhibiting significant *in vitro* inhibition of human transthyretin were tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 625 nM, 1250 nM, 2500 nM, 5000 nM and 10000 nM concentrations of antisense oligonucleotide, as specified in Table 5. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS3029 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells.

[0355] The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 5 and was calculated by plotting the concentrations of oligonucleotides used versus the percent inhibition of transthyretin mRNA expression achieved at each concentration, and noting the concentration of oligonucleotide at which 50% inhibition of transthyretin mRNA expression was achieved compared to the control. As illustrated in Table 5, transthyretin mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 5

Dose-dependent antisense inhibition of human transthyretin in HepG2 cells using electroporation						
ISIS NO	625 nM	1250 nM	2500 nM	5000 nM	10000 nM	IC_{50} (μM)
304296	57	74	83	91	96	<0.625
304299	43	76	82	95	94	0.627
420913	59	75	90	88	98	<0.625
420915	60	85	91	95	99	<0.625
420951	64	77	90	97	99	<0.625
425653	70	86	86	88	82	<0.625
425655	48	80	85	97	96	<0.625
425656	70	89	92	92	96	<0.625
425659	46	56	68	82	93	0.8
425679	63	77	72	94	97	<0.625
425680	28	79	85	93	98	0.8
425693	2	64	74	76	81	1.7
425695	74	87	91	97	98	<0.625
425716	69	84	95	97	98	<0.625
425719	58	84	92	96	98	<0.625
425721	40	75	89	95	98	0.7
425736	64	71	86	93	93	<0.625
425737	78	93	95	97	98	<0.625
425738	40	77	88	94	95	0.7
425754	56	75	87	96	99	<0.625
425755	58	84	88	94	97	<0.625
425757	62	82	94	97	99	<0.625
425760	58	42	74	85	93	<0.625
425765	81	86	87	83	88	<0.625
425766	83	89	81	75	74	<0.625
425767	56	75	83	81	80	<0.625

[0356] Gapmers from Example 2 were also tested at various doses in HepG2 cells using the transfection reagent, lipofectin. Cells were plated at a density of 10,000 cells per well and transfected using electroporation with 6.25 nM, 12.5 nM, 25 nM, 50 nM and 100 nM concentrations of antisense oligonucleotide, as specified in Table 6. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS3029 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illustrated in Table 6, transthyretin mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 6

Dose-dependent antisense inhibition of human transthyretin in HepG2 cells using lipofectin reagent						
ISIS NO	6.25 nM	12.5 nM	25 nM	50 nM	100 nM	IC_{50} (nM)
304296	26	41	43	52	65	39
304299	22	70	43	74	83	20
420913	4	60	60	68	82	30
420915	36	31	46	64	67	28
420951	10	37	56	85	84	19
425653	25	38	60	74	77	18
425655	27	15	62	79	81	16
425656	37	62	47	69	82	15
425659	17	35	33	79	73	30
425679	32	6	63	79	77	14
425680	16	48	41	84	84	28
425693	10	19	51	66	61	26
425695	36	23	54	76	84	28
425716	57	52	36	85	81	38
425719	25	39	28	60	76	45
425721	0	22	38	73	75	32
425736	25	60	30	77	80	22
425737	36	52	50	60	76	14
425738	13	15	19	65	70	27
425754	8	18	38	75	71	42
425755	26	46	54	77	86	20
425757	0	37	81	83	71	19
425760	28	46	72	70	80	18
425765	0	52	48	66	69	29
425766	24	19	48	69	71	29
425767	41	49	48	65	75	14

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

[0357] Gapmers selected from Example 3 were tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.0617 μM, 0.1852 μM, 0.5556 μM, 1.6667 μM and 5 μM concentrations of antisense oligonucleotide, as specified in Table 7. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR.

Human transthyretin primer probe set RTS3029 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illustrated in Table 7, transthyretin mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 7

Dose-dependent antisense inhibition of human transthyretin in HepG2 cells using electroporation						
ISIS NO	0.0617 μM	0.1852 μM	0.5556 μM	1.6667 μM	5 μM	IC_{50} (μM)
304296	0	6	44	58	83	1.2
304299	38	10	57	83	92	0.6
420913	51	51	54	73	93	0.2
420915	33	35	62	65	93	0.2
420951	40	33	36	82	96	0.4
425653	55	58	74	72	84	< 0.06
425655	8	35	54	57	90	0.5
425656	12	43	43	78	94	0.4
425659	14	35	19	46	82	0.6
425679	30	13	23	69	91	0.8
425680	0	35	45	74	84	0.7
425693	0	6	14	32	59	3.4
425695	15	47	61	81	91	0.3
425716	20	17	53	77	91	0.6
425719	0	14	45	78	94	0.8
425721	0	0	22	74	84	0.9
425736	42	43	56	76	91	0.3
425737	21	29	61	81	97	0.3
425738	14	39	57	74	93	0.4
425754	29	34	45	78	94	0.4
425755	8	21	57	78	95	0.5
425757	29	28	62	83	95	0.4
425760	3	6	9	56	77	1.4
425765	24	51	75	77	88	0.3
425766	7	41	59	73	77	0.3
425767	1	18	49	66	79	1.0

Example 5: Dose response confirmation of antisense oligonucleotides targeting human transthyretin in Hep3B cells

[0358] Gapmers from Example 4 exhibiting significant *in vitro* inhibition of human transthyretin 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 0.0206 μM, 0.062 μM, 0.185 μM, 0.556 μM, 1.667 μM and 5 μM concentrations of antisense oligonucleotide, as specified in Table 8. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS 1396 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illus-

trated in Table 8, transthyretin mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. The IC_{50} of each oligonucleotide is also presented in Table 8.

Table 8

Dose-dependent antisense inhibition of human transthyretin in Hep3B cells using electroporation							
ISIS NO	0.0206 μM	0.062 μM	0.185 μM	0.556 μM	1.667 μM	5 μM	IC_{50} (μM)
304299	27	2	25	52	76	96	0.5
420915	0	12	27	30	69	93	0.8
425653	23	13	55	86	88	91	0.1
425655	3	30	32	62	84	94	0.3
425656	0	0	29	66	82	95	0.5
425679	0	21	36	71	92	97	0.3
425695	37	23	63	79	94	98	0.1
425736	31	43	40	64	82	95	0.1
425737	0	13	62	82	95	98	0.2
425755	17	8	18	69	86	98	0.4
425757	22	47	53	79	96	98	0.2

Example 6: Dose response confirmation of antisense oligonucleotides targeting human transthyretin in human transthyretin-transgenic mouse primary hepatocytes

[0359] Gapmers from Example 5 were also tested at various doses in primary hepatocytes of human transthyretin-transgenic mice. ISIS 304309, ISIS 304311, ISIS 304312 and ISIS 420951 (see Example 2) were also retested along with these gapmers under the same culture conditions. Cells were plated at a density of 10,000 cells per well and transfected using cytofectin with 18.75 nM, 37.5 nM, 75 nM, 150 nM and 300 nM concentrations of antisense oligonucleotide, as specified in Table 9. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS1396 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illustrated in Table 9, transthyretin mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 9

Dose-dependent antisense inhibition of human transthyretin in mouse primary hepatocytes using cytofectin						
ISIS NO	18.75 nM	37.5 nM	75 nM	150 nM	300 nM	Motif
304299	54	79	97	98	99	5-10-5
304309	48	77	94	99	99	5-10-5
304311	45	79	92	96	98	5-10-5
304312	33	71	89	96	98	5-10-5
420915	40	70	92	98	99	5-10-5
420951	41	86	96	98	99	5-10-5
425653	44	81	93	96	99	5-10-5
425655	61	88	96	99	99	3-14-3
425656	61	84	94	98	99	3-14-3
425679	74	78	97	98	99	3-14-3
425695	66	84	96	98	99	2-13-5

(continued)

Dose-dependent antisense inhibition of human transthyretin in mouse primary hepatocytes using cytofectin						
ISIS NO	18.75 nM	37.5 nM	75 nM	150 nM	300 nM	Motif
425736	58	84	95	98	99	4-11-5
425737	57	77	95	98	99	4-11-5
425755	61	82	96	99	99	4-11-5
425757	37	77	93	98	98	4-11-5

Example 7: Dose response confirmation of antisense oligonucleotides targeting human transthyretin in HepG2 cells

[0360] Gapmers from Example 6 were tested at various doses in HepG2 cells. Cells were plated at a density of 10,000 cells per well and transfected using electroporation with 0.062 μM, 0.185 μM, 0.556 μM, 1.66 μM and 5000 μM concentrations of antisense oligonucleotide, as specified in Table 10. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS1396 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illustrated in Table 10, transthyretin mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 10

Dose-dependent antisense inhibition of human transthyretin in HepG2 cells using electroporation							
ISIS NO	0.062 μM	0.185 μM	0.556 μM	1.667 μM	5.000 μM	IC_{50} (μM)	Motif
304299	55	66	72	87	96	0.037	5-10-5
304309	41	65	72	91	96	0.087	5-10-5
304311	57	83	88	89	83	0.001	5-10-5
304312	46	69	74	84	81	0.038	5-10-5
420915	38	62	80	90	98	0.096	5-10-5
420951	45	71	84	93	97	0.049	5-10-5
425653	48	73	87	88	82	0.017	5-10-5
425655	40	57	77	85	95	0.105	3-14-3
425656	28	54	70	94	97	0.177	3-14-3
425679	43	51	81	95	99	0.106	3-14-3
425695	49	67	90	96	99	0.043	2-13-5
425736	32	63	85	95	98	0.108	4-11-5
425737	42	71	90	98	99	0.053	4-11-5
425755	24	63	85	95	99	0.137	4-11-5
425757	21	62	86	96	99	0.148	4-11-5

Example 8: Dose response confirmation of antisense oligonucleotides targeting human transthyretin in human transthyretin-transgenic mouse primary hepatocytes

[0361] Gapmers from Example 6 were also tested at various doses in primary hepatocytes of human transthyretin-transgenic mice. Cells were plated at a density of 10,000 cells per well and transfected using cytofectin with 5 nM, 10 nM, 20 nM, 40 nM and 80 nM concentrations of antisense oligonucleotide, as specified in Table 11. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by

quantitative real-time PCR. Human transthyretin primer probe set RTS3029 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illustrated in Table 11, transthyretin mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 11

Dose-dependent antisense inhibition of human transthyretin in mouse primary hepatocytes using cytofectin						
ISIS NO	5 nM	10 nM	20 nM	40 nM	80 nM	Motif
304299	0	8	37	69	90	5-10-5
304309	0	9	39	75	93	5-10-5
304311	1	13	43	70	81	5-10-5
304312	0	3	32	64	76	5-10-5
420915	0	0	34	59	87	5-10-5
420951	0	12	57	84	92	5-10-5
425653	0	9	44	72	84	5-10-5
425655	0	19	45	80	91	3-14-3
425656	0	2	33	70	93	3-14-3
425679	0	13	42	72	90	3-14-3
425695	3	12	33	70	90	2-13-5
425736	2	7	37	70	89	4-11-5
425737	0	4	36	65	89	4-11-5
425755	0	25	50	75	94	4-11-5
425757	0	5	43	72	92	4-11-5

[0362] Gapmers were also tested using electroporation as the transfection agent. Cells were plated at a density of 35,000 cells per well and transfected using electroporation with 148.148 nM, 444.444 nM, 1,333.333 nM, 4,000 nM and 12,000 nM concentrations of antisense oligonucleotide, as specified in Table 12. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS3029 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells.

Table 12

Dose-dependent antisense inhibition of human transthyretin in mouse primary hepatocytes using electroporation						
ISIS NO	148.148 nM	444.444 nM	1333.333 nM	4000 nM	12000 nM	Motif
304299	75	96	98	98	99	5-10-5
304309	72	96	98	98	98	5-10-5
304311	68	92	93	94	97	5-10-5
304312	50	84	92	93	97	5-10-5
420915	55	89	96	96	97	5-10-5
420951	65	92	95	96	98	5-10-5
425653	68	89	91	93	95	5-10-5
425655	63	94	96	96	96	3-14-3
425656	69	93	98	98	98	3-14-3

(continued)

Dose-dependent antisense inhibition of human transthyretin in mouse primary hepatocytes using electroporation						
ISIS NO	148.148 nM	444.444 nM	1333.333 nM	4000 nM	12000 nM	Motif
425679	63	92	97	98	98	3-14-3
425695	69	92	96	96	95	2-13-5
425736	75	93	96	96	96	4-11-5
425737	71	94	96	96	95	4-11-5
425755	70	93	95	95	95	4-11-5
425757	61	91	95	95	95	4-11-5

Example 9: Dose response confirmation of antisense oligonucleotides targeting human transthyretin in cynomolgus monkey primary hepatocytes

[0363] Gapmers from Example 6 were also tested at various doses in primary hepatocytes of cynomolgus monkeys. Cells were plated at a density of 35,000 cells per well and transfected using electroporation with 1,250 nM, 2,500 nM, 5,000 nM, 10,000 nM and 20,000 nM concentrations of antisense oligonucleotide, as specified in Table 13. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS1396 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illustrated in Table 13, transthyretin mRNA levels were reduced in a dose-dependent manner in hepatocytes treated with ISIS oligonucleotides.

[0364] In absence of a complete cynomolgus monkey gene sequence in the NCBI database, the oligonucleotides were tested for cross-reactivity against the rhesus monkey gene sequence, since the two species are from the same genus, 'Macaca'. The human oligonucleotides are cross-reactive with rhesus monkey transthyretin gene, designated herein as SEQ ID NO: 4 (exons 1-4 extracted from GENBANK Accession No. NW_001105671.1). 'Mismatches' indicates the number of mismatches between the human oligonucleotide and the rhesus monkey transthyretin gene. 'n/a' indicates that the human oligonucleotide has more than 3 mismatches with the rhesus monkey transthyretin gene and therefore does not cross-react with it.

Table 13

Dose-dependent antisense inhibition of human transthyretin in Rhesus monkey primary hepatocytes using electroporation									
ISIS NO	1,250 nM	2,500 nM	5,000 nM	10,000 nM	2,0000 nM	IC_{50} (μM)	Rhesus monkey Target start site	Rhesus monkey Target stop site	Mis matches
304299	21	45	69	80	95	3.1	504	523	0
304309	53	66	79	85	93	<1.25	575	594	0
304311	75	78	82	86	90	<1.25	594	613	0
304312	37	53	65	75	80	2.3	595	614	0
420915	59	54	77	87	94	<1.25	505	524	0
420951	67	77	91	93	96	<1.25	578	597	0
425653	56	72	84	83	85	<1.25	593	612	0
425655	0	7	0	21	45	>20	478	497	2
425656	41	20	38	53	51	8.7	479	498	2
425679	68	74	88	94	98	<1.25	578	597	0
425695	42	29	41	49	65	25.8	478	497	2

(continued)

Dose-dependent antisense inhibition of human transthyretin in Rhesus monkey primary hepatocytes using electroporation									
ISIS NO	1,250 nM	2,500 nM	5,000 nM	10,000 nM	2,0000 nM	IC_{50} (μM)	Rhesus monkey Target start site	Rhesus monkey Target stop site	Mis matches
425736	36	27	37	49	74	8.2	479	498	2
425737	76	78	89	95	97	<1.25	501	520	0
425755	79	80	92	94	97	<1.25	578	597	0
425757	68	74	88	95	96	<1.25	580	599	0

Example 10: *In vivo* inhibition of human transthyretin in human transthyretin-transgenic mice

[0365] Gapmers from Example 6, demonstrating significant inhibition of transthyretin mRNA, were tested in transgenic mice containing the human transthyretin gene and the efficacy of the gapmers was evaluated.

Treatment

[0366] Fifteen groups of four hTTR transgenic female mice each were administered subcutaneously twice a week for four weeks with 25 mg/kg of ISIS 304299, ISIS 304309, ISIS 304311, ISIS 304312, ISIS 420915, ISIS 420951, ISIS 425653, ISIS 425655, ISIS 425656, ISIS 425679, ISIS 425695, ISIS 425736, ISIS 425737, ISIS 425755, or ISIS 425757. Another group of four female hTTR transgenic mice was injected with 25 mg/kg of control oligonucleotide ISIS 141923 (CCTTCCCTGAAGGTTCCTCC, designated herein as SEQ ID NO: 165) twice a week for four weeks. Another group of four hTTR transgenic female mice were injected subcutaneously with PBS twice a week for four weeks. The mice injected with PBS served as a control group. Blood samples were collected from all groups on weeks 0, 1, 2, 3, and 4 for plasma transthyretin level analysis. The mice were sacrificed two days after the last dose and livers were harvested for target mRNA analysis.

RNA analysis

[0367] RNA was extracted from liver tissue for real-time PCR analysis of transthyretin using primer probe set RTS3029. Results are presented as percent inhibition of human transthyretin, relative to PBS control. As shown in Table 14, treatment with ISIS antisense oligonucleotides resulted in significant reduction of human transthyretin mRNA in comparison to the PBS control. Treatment with the control oligonucleotide, ISIS 141923 did not result in significant reduction of transthyretin, as expected.

Table 14

Inhibition of human transthyretin mRNA in the hTTR transgenic mice liver relative to the PBS control	
ISIS NO	% inhibition
304299	79
304309	83
304311	63
304312	64
420915	82
420951	92
425653	66
425655	76
425656	76

(continued)

Inhibition of human transthyretin mRNA in the hTTR transgenic mice liver relative to the PBS control	
ISIS NO	% inhibition
425679	93
425695	82
425736	63
425737	76
425755	91
425757	91
141923	28

Protein analysis

[0368] Human transthyretin protein levels were measured in transgenic mice plasma by ELISA using an anti-transthyretin polyclonal antibody (Abcam Ab37774) and a sheep anti-TTR horse radish peroxidase detection antibody (Abcam cat. no. 35217). The color reaction was developed by the ImmunoPure® TMB Substrate Kit and absorbance measured at 450 nm using a microtiter plate spectrophotometer. Plasma samples were taken predose and on days 7, 14 and 28. The results are presented in Table 15 expressed as percentage inhibition compared to the predose levels and demonstrate a time-dependent reduction in protein levels with treatment with ISIS oligonucleotides.

Table 15

Inhibition of human transthyretin protein in the hTTR transgenic mice plasma relative to predose levels									
	PBS	ISIS 304299	ISIS 304309	ISIS 420915	ISIS 420951	ISIS 425679	ISIS 425695	ISIS 425755	ISIS 141923
Day 7	0	50	63	71	92	99	69	57	3
Day 14	3	76	78	90	98	100	80	72	3
Day 21	20	88	81	95	100	99	88	78	13
Day 28	13	89	83	98	100	100	91	79	8

Body weight and organ weight

[0369] The body weights of the mice were measured predose and at the end of the treatment period. The body weights are presented in Table 16 and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 16 as a percent change over the respective organ weights of the PBS control. As shown in Table 16, there was no significant change in body or organ weights as a result of antisense oligonucleotide treatment.

Table 16

Percent change in body and organ weights of transgenic mice after antisense oligonucleotide treatment				
	Body weight	Liver	Spleen	Kidney
PBS	1.1	1.0	1.0	1.0
ISIS 304299	1.1	1.1	1.0	0.8
ISIS 304309	1.1	1.1	1.0	1.0
ISIS 304311	1.1	1.2	1.0	1.2
ISIS 304312	1.1	1.3	1.0	0.8
ISIS 420915	1.1	1.1	1.0	1.1

(continued)

Percent change in body and organ weights of transgenic mice after antisense oligonucleotide treatment				
	Body weight	Liver	Spleen	Kidney
ISIS 420951	1.1	1.2	1.0	1.5
ISIS 425653	1.1	1.1	0.9	1.0
ISIS 425655	1.1	1.3	1.0	1.2
ISIS 425656	1.2	1.3	1.0	1.3
ISIS 425679	1.2	1.2	1.0	1.6
ISIS 425695	1.1	1.3	1.0	1.0
ISIS 425736	1.2	1.2	1.0	1.0
ISIS 425737	1.1	1.2	1.1	1.2
ISIS 425755	1.2	1.3	1.1	1.3
ISIS 425757	1.1	1.9	1.0	1.5
ISIS 141923	1.1	1.1	1.0	0.8

Liver function

[0370] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 17, expressed in IU/L. Plasma levels of bilirubin were also measured using the same clinical chemistry analyzer; results are also presented in Table 17 and expressed in mg/dL.

Table 17

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of transgenic mice			
	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)
PBS	31	78	0.23
ISIS 304299	40	121	0.19
ISIS 304309	38	119	0.20
ISIS 304311	34	60	0.16
ISIS 304312	43	67	0.17
ISIS 420915	34	75	0.26
ISIS 420951	75	124	0.17
ISIS 425653	35	78	0.20
ISIS 425655	131	109	0.16
ISIS 425656	68	110	0.19
ISIS 425679	119	180	0.20
ISIS 425695	43	69	0.15
ISIS 425736	23	58	0.16
ISIS 425737	35	64	0.19
ISIS 425755	109	162	0.16
ISIS 425757	1904	937	0.24
ISIS 141923	31	76	0.19

Kidney function

[0371] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 18, expressed in mg/dL. The data indicates that antisense inhibition of transthyretin has no effect on BUN levels in these transgenic mice.

Table 18

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of transgenic mice	
	BUN (mg/dL)
PBS	26
ISIS 304299	24
ISIS 304309	29
ISIS 304311	28
ISIS 304312	26
ISIS 420915	25
ISIS 420951	25
ISIS 425653	24
ISIS 425655	28
ISIS 425656	25
ISIS 425679	26
ISIS 425695	28
ISIS 425736	25
ISIS 425737	23
ISIS 425755	24
ISIS 425757	25
ISIS 141923	23

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

[0372] CD1® mice (Charles River, MA) are a multipurpose model of mice, frequently utilized for safety and efficacy testing. The mice were treated with ISIS antisense oligonucleotides selected from studies described in Example 10 and evaluated for changes in the levels of various metabolic markers.

Treatment

[0373] Groups of eight CD1 mice each were injected subcutaneously twice a week with 50 mg/kg of ISIS 304299, ISIS 304309, ISIS 420915, ISIS 420951, ISIS 425655, ISIS 425656, ISIS 425679, ISIS 425695, ISIS 425736, ISIS 425737, and ISIS 425755. Four mice from each group were evaluated at week 2 and week 6 of the treatment period. Three days after the last dose at each time point, body weights were taken, mice were euthanized and organs and plasma were harvested for further analysis.

Body and organ weights

[0374] The body weights of the mice were measured pre-dose and at the end of each treatment period (two weeks and six weeks). The body weights are presented in Tables 19 and 20, and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Tables 19 and 20 as a percentage change over the respective organ weights of the PBS control.

Table 19

Change in body and organ weights of CD1 mice after antisense oligonucleotide treatment (%) at week 2				
	Body weight	Liver	Spleen	Kidney
PBS	1.1	1.0	1.0	1.0
ISIS 304299	1.1	1.1	1.1	1.1
ISIS 304309	1.1	1.1	1.1	1.0
ISIS 420915	1.1	1.1	1.1	1.0
ISIS 420951	1.1	1.3	1.7	1.2
ISIS 425655	1.1	1.2	1.2	0.9
ISIS 425656	1.1	1.1	1.1	1.0
ISIS 425679	1.1	1.1	1.4	1.1
ISIS 425695	1.1	1.1	0.9	1.1
ISIS 425736	1.1	1.1	1.0	1.1
ISIS 425737	1.2	1.1	1.1	1.1
ISIS 425755	1.2	1.2	1.3	1.2

Table 20

Change in body and organ weights of CD1 mice after antisense oligonucleotide treatment (%) at week 6				
	Body weight	Liver	Spleen	Kidney
PBS	1.2	1.0	1.0	1.0
ISIS 304299	1.3	1.2	1.4	1.0
ISIS 304309	1.3	1.3	2.0	1.0
ISIS 420915	1.3	1.1	1.5	0.9
ISIS 420951	1.3	1.3	2.0	1.1
ISIS 425655	1.4	1.3	1.7	0.9
ISIS 425656	1.3	1.3	1.1	1.0
ISIS 425679	1.3	1.4	2.3	1.2
ISIS 425695	1.3	1.4	1.5	1.0
ISIS 425736	1.3	1.1	1.2	0.9
ISIS 425737	1.2	1.1	1.3	1.0
ISIS 425755	1.3	1.3	2.1	1.0

Liver function

[0375] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Tables 21 and 22 expressed in IU/L. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer and the results are also presented in Tables 21 and 22.

Table 21

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of CD1 mice at week 2				
	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (g/dL)
PBS	38	66	0.19	5.0
ISIS 304299	42	79	0.33	3.8
ISIS 304309	52	77	0.22	3.2
ISIS 420915	32	61	0.28	3.5
ISIS 420951	1184	804	0.17	3.7
ISIS 425655	60	70	0.20	3.9
ISIS 425656	37	53	0.31	3.5
ISIS 425679	88	147	0.23	3.7
ISIS 425695	25	50	0.23	3.6
ISIS 425736	31	79	0.23	3.2
ISIS 425737	39	43	0.23	3.1
ISIS 425755	104	85	0.29	3.6

Table 22

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of CD1 mice at week 6				
	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (g/dL)
PBS	31	67	0.20	5.6
ISIS 304299	54	71	0.20	5.2
ISIS 304309	1211	504	0.30	5.2
ISIS 420915	89	91	0.17	5.0
ISIS 420951	872	319	0.20	3.6
ISIS 425655	730	247	0.13	4.3
ISIS 425656	502	261	0.17	4.3
ISIS 425679	935	475	0.29	4.5
ISIS 425695	1627	563	0.16	4.0
ISIS 425736	41	47	0.15	4.1
ISIS 425737	32	55	0.16	4.1
ISIS 425755	233	176	0.16	4.3

Kidney function

[0376] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Tables 23 and 24, expressed in mg/dL.

Table 23

Effect of antisense oligonucleotide treatment on metabolic markers (mg/dL) in the kidney of CD1 mice at week 2		
	BUN	Creatinine
PBS	32	0.23

(continued)

Effect of antisense oligonucleotide treatment on metabolic markers (mg/dL) in the kidney of CD1 mice at week 2		
	BUN	Creatinine
ISIS 304299	26	0.21
ISIS 304309	30	0.19
ISIS 420915	30	0.22
ISIS 420951	24	0.17
ISIS 425655	29	0.22
ISIS 425656	25	0.19
ISIS 425679	28	0.19
ISIS 425695	29	0.19
ISIS 425736	24	0.19
ISIS 425737	24	0.16
ISIS 425755	27	0.17

Table 24

Effect of antisense oligonucleotide treatment on metabolic markers (mg/dL) in the kidney of CD1 mice at week 6		
	BUN	Creatinine
PBS	24	0.15
ISIS 304299	19	0.11
ISIS 304309	20	0.14
ISIS 420915	24	0.18
ISIS 420951	19	0.08
ISIS 425655	22	0.11
ISIS 425656	21	0.10
ISIS 425679	20	0.06
ISIS 425695	21	0.08
ISIS 425736	22	0.07
ISIS 425737	18	0.07
ISIS 425755	22	0.09

Hematology assays

[0377] Blood obtained from all mice groups were sent to Antech Diagnostics for hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) measurements and analyses, as well as measurements of the differential blood cell counts, such as that of WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 25-28. Percentages given in the tables indicate the percent change in total blood cell count compared to the PBS control. Those antisense oligonucleotides which did not affect a decrease in platelet count less than 70% of the PBS control or an increase in monocyte count more than two-fold were selected for further studies.

Table 25

Effect of antisense oligonucleotide treatment on complete blood cell count (%) compared to the PBS control in CD1 mice at week 2							
ISIS NO.	WBC	RBC	Hemoglobin	HCT	MCV	MCH	MCHC
304299	-15	-3	-2	0	+3	+1	-1
304309	-13	-4	-7	-6	-2	-4	-2
420915	+7	-7	-7	-5	+2	+1	-2
420951	+79	-6	-5	-5	+1	+1	0
425655	+56	-3	-5	-4	-1	-2	-1
425656	+69	-5	-6	-5	0	-1	-2
425679	+30	-6	-7	-7	-1	-1	0
425695	+49	-3	-4	-4	0	0	+1
425736	+15	-6	-6	-4	+1	0	-2
425737	+19	-5	-7	-5	-1	-3	-2
425755	+85	-3	-6	-6	-4	-3	0

Table 26

Effect of antisense oligonucleotide treatment on complete blood cell count (%) compared to the PBS control in CD1 mice at week 6							
ISIS NO.	WBC	RBC	Hemoglobin	HCT	MCV	MCH	MCHC
304299	-7	-9	-10	-13	-5	0	+4
304309	+10	-12	-11	-15	-5	+1	+6
420915	+11	-7	-8	-10	-4	-2	+2
420951	+81	-12	-20	-19	-9	-9	-1
425655	+29	-3	-11	-10	-8	-9	-2
425656	+72	-1	-5	-6	-4	-5	-1
425679	+154	-11	-20	-21	-10	-9	+2
425695	+118	+3	-9	-9	-2	-12	+3
425736	+51	+4	-5	-7	0	-10	+1
425737	+30	+8	-1	-2	0	-8	+1
425755	+54	-1	-11	-12	-8	-10	0

Table 27

Effect of antisense oligonucleotide treatment on differential blood cell count (%) compared to the PBS control in CD1 mice at week 2				
ISIS NO.	Neutrophils	Monocytes	Lymphocytes	Platelets
304299	11	-3	20	17
304309	-11	5	8	14
420915	1	4	-24	41
420951	18	-7	32	-9

(continued)

Effect of antisense oligonucleotide treatment on differential blood cell count (%) compared to the PBS control in CD1 mice at week 2				
ISIS NO.	Neutrophils	Monocytes	Lymphocytes	Platelets
425655	18	-5	20	18
425656	31	-7	-4	24
425679	2	-1	24	-19
425695	-50	15	20	29
425736	8	-1	0	10
425737	-29	10	-8	24
425755	-13	7	-4	9

Table 28

Effect of antisense oligonucleotide treatment on differential blood cell count (%) compared to the PBS control in CD1 mice at week 6				
ISIS NO.	Neutrophils	Lymphocytes	Monocytes	Platelets
304299	-60	+26	+10	-16
304309	-28	+12	+30	+2
420915	-29	+6	+50	-30
420951	-26	+11	0	-40
425655	-16	+8	-10	-19
425656	-22	+16	-50	-25
425679	-36	+19	-20	-27
425695	-25	+9	-15	-49
425736	-41	+16	-5	-46
425737	-53	+23	-20	-65
425755	-20	+4	+25	-41

Example 12: Measurement of half-life of antisense oligonucleotide in CD1 mouse liver

[0378] CD1 mice were treated with ISIS antisense oligonucleotides from studies described in Example 11 and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver was evaluated.

Treatment

[0379] Groups of twelve CD1 mice each were injected subcutaneously twice per week for 2 weeks with 50 mg/kg of ISIS 304299, ISIS 304309, ISIS 420915, ISIS 420951, ISIS 425655, ISIS 425656, ISIS 425679, ISIS 425695, ISIS 425736, ISIS 425737, and ISIS 425755. Four mice from each group were sacrificed 3 days, 28 days and 56 days following the final dose. Livers were harvested for analysis.

Measurement of oligonucleotide concentration

[0380] The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenolchloroform (liquid-liquid) extraction followed by a solid phase extraction.

An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTT-GCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 166) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. Half-lives were then calculated using WinNonlin software (PHARSIGHT).

[0381] The results are presented in Tables 29 and 30, expressed as μg/g liver tissue. The half-life of each oligonucleotide is presented in Table 31. Antisense oligonucleotides with half-lives within 11-34 days were chosen for further studies.

Table 29

Full-length oligonucleotide concentration (μg/g) in the liver of CD1 mice			
ISIS NO.	3 days	28 days	56 days
304299	180	56	8
304309	317	254	106
420915	248	126	34
420951	173	109	49
425655	191	113	33
425656	256	73	29
425679	201	73	27
425695	315	194	65
425736	219	110	47
425737	190	40	9
425755	211	120	47

Table 30

Total oligonucleotide concentration (μg/g) in the liver of CD1 mice			
ISIS NO.	3 days	28 days	56 days
304299	268	168	38
304309	389	354	152
420915	314	229	83
420951	262	196	131
425655	298	217	87
425656	328	135	85
425679	333	161	103
425695	364	263	143
425736	298	211	140
425737	266	117	31
425755	337	227	140

Table 31

Half-life of oligonucleotide (days) in the liver of CD1 mice	
ISIS NO.	Half-life (days)
304299	12
304309	33
420915	19
420951	29
425655	21
425656	17
425679	18
425695	23
425736	24
425737	12
425755	24

Example 13: Tolerability of antisense oligonucleotides targeting human transthyretin in Sprague-Dawley rats

[0382] Sprague-Dawley rats were treated with ISIS antisense oligonucleotides selected from studies described in Examples 11 and 12 and evaluated for changes in the levels of various metabolic markers.

Treatment

[0383] The body weights, complete blood count and different blood count, as well as the urine protein/ creatinine ratio of the rats were evaluated pre-dose. Groups of four Sprague-Dawley rats each were injected subcutaneously twice a week with 50 mg/kg of ISIS 304299, ISIS 304309, ISIS 420915, ISIS 420951, ISIS 425655, ISIS 425656, ISIS 425679, ISIS 425695, ISIS 425736, ISIS 425737, and ISIS 425755. Three days after the last dose at each time point, body weights were taken, mice were euthanized and organs and plasma were harvested for further analysis.

Body and organ weights

[0384] The body weights of the rats were measured pre-dose and at the end of the treatment period. The body weights are presented in Table 32, and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 32 as a percentage change over the respective organ weights of the PBS control.

Table 32

Change in body and organ weights of Sprague-Dawley rats after antisense oligonucleotide treatment (%)				
	Body weight	Liver	Spleen	Kidney
PBS	1.6	1.0	1.0	1.0
ISIS 304299	1.2	1.7	4.9	1.6
ISIS 304309	1.1	1.6	4.3	1.4
ISIS 420915	1.4	1.4	3.3	1.3
ISIS 420951	1.1	1.4	5.0	1.5
ISIS 425655	1.2	1.5	3.4	1.3
ISIS 425656	1.2	1.5	2.9	1.2
ISIS 425679	1.0	1.9	6.4	1.7

(continued)

Change in body and organ weights of Sprague-Dawley rats after antisense oligonucleotide treatment (%)				
	Body weight	Liver	Spleen	Kidney
ISIS 425695	1.2	1.6	3.3	1.3
ISIS 425736	1.3	1.5	2.9	1.2
ISIS 425737	1.2	1.7	4.0	1.5
ISIS 425755	1.0	1.5	5.4	1.5

[0385] As shown in Tables 32, certain compounds showed a less than a 4-fold increase in spleen weight.

Liver function

[0386] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 33 expressed in IU/L. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer and the results are also presented in Table 33.

Table 33

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of Sprague-Dawley rats				
	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Albumin (g/dL)
PBS	55	138	0.15	3.3
ISIS 304299	69	154	0.15	2.7
ISIS 304309	80	138	0.11	2.9
ISIS 420915	43	95	0.11	3.0
ISIS 420951	353	511	0.32	2.6
ISIS 425655	312	497	0.47	2.6
ISIS 425656	277	335	0.20	3.0
ISIS 425679	537	659	0.38	2.7
ISIS 425695	228	445	0.23	2.3
ISIS 425736	362	553	0.32	2.9
ISIS 425737	55	79	0.09	1.9
ISIS 425755	271	303	0.41	2.8

Kidney function

[0387] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 34, expressed in mg/dL. The ratio of total urine protein to creatinine was also evaluated and presented in Table 35.

Table 34

Effect of antisense oligonucleotide treatment on metabolic markers (mg/dL) in the kidney of Sprague-Dawley rats		
	BUN	Creatinine
PBS	20	0.26
ISIS 304299	30	0.40

(continued)

Effect of antisense oligonucleotide treatment on metabolic markers (mg/dL) in the kidney of Sprague-Dawley rats		
	BUN	Creatinine
ISIS 304309	24	0.33
ISIS 420915	20	0.26
ISIS 420951	37	0.47
ISIS 425655	28	0.40
ISIS 425656	25	0.34
ISIS 425679	46	0.42
ISIS 425695	30	0.37
ISIS 425736	26	0.37
ISIS 425737	30	0.36
ISIS 425755	29	0.36

Table 35

Effect of antisense oligonucleotide treatment on total urine protein/ creatinine in the kidney of Sprague-Dawley rats		
	Pre-dose	Week 6
PBS	0.82	0.95
ISIS 304299	0.95	7.57
ISIS 304309	1.10	5.20
ISIS 420915	0.91	5.30
ISIS 420951	0.90	5.02
ISIS 425655	0.78	6.03
ISIS 425656	0.86	9.37
ISIS 425679	0.91	7.80
ISIS 425695	0.89	5.71
ISIS 425736	1.00	5.85
ISIS 425737	0.86	43.76
ISIS 425755	0.78	3.70

[0388] As shown in Tables 34 and 35, certain compounds demonstrated a less than 7-fold increase in the total urine protein/creatinine in the kidney of these rats. Furthermore, certain compounds demonstrated a less than 6-fold increase in the total urine protein/creatinine in the kidney of these rats..

Hematology assays

[0389] Blood obtained from all rat groups were sent to Antech Diagnostics for hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) measurements and analyses, as well as measurements of the differential blood cell counts, such as that of WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 36 and 37. Percentages given in the tables indicate the percent change in total blood cell count compared to the PBS control.

Table 36

Effect of antisense oligonucleotide treatment on complete blood cell count (%) compared to the PBS control in Sprague-Dawley rats							
ISIS NO.	WBC	RBC	Hemoglobin	HCT	MCV	MCH	MCHC
304299	+4	-5	-3	+2	+11	+5	-5
304309	-10	-8	-11	-12	-4	-3	+1
420915	-9	-16	-20	-17	+1	-3	-3
420951	+5	-5	-8	-5	+1	-2	-3
425655	+22	-17	-18	-19	-2	0	+2
425656	-1	-13	-19	-16	-3	-6	-2
425679	+49	-42	-32	-28	+26	+19	-5
425695	-2	-25	-31	-29	-4	-8	-3
425736	+18	+1	-3	+2	0	-4	-4
425737	-15	-20	-18	-20	+2	+3	+1
425755	+35	-31	-27	-23	+14	+8	-4

Table 37

Effect of antisense oligonucleotide treatment on complete blood cell count (%) compared to the PBS control in Sprague-Dawley rats				
ISIS NO.	Neutrophils	Lymphocytes	Monocytes	Platelet
304299	-61	+15	-10	-41
304309	-35	+8	+10	-37
420915	-23	+6	0	-29
420951	-62	+15	+10	-67
425655	+23	-8	+80	-13
425656	-14	0	+70	-15
425679	-4	-1	+60	-75
425695	+68	-20	+80	-5
425736	0	-2	+70	-1
425737	-6	+1	+20	-21
425755	-18	+3	+70	-58

Example 14: Pharmacokinetic studies of antisense oligonucleotide concentration in Sprague-Dawley rat liver and kidney

[0390] Sprague Dawley rats were treated with ISIS antisense oligonucleotides from studies described in Example 13 and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver and kidney was evaluated.

Treatment

[0391] Groups of four Sprague Dawley rats each were injected subcutaneously twice a week for 2 weeks with 20 mg/kg of ISIS 304299, ISIS 304309, ISIS 420915, ISIS 420951, ISIS 425655, ISIS 425656, ISIS 425679, ISIS 425695, ISIS 425736, ISIS 425737, and ISIS 425755. Three days after the last dose, the rats were sacrificed and livers and

kidneys were collected for analysis.

Measurement of oligonucleotide concentration

[0392] The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenolchloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTT-GCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 166) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. The results are presented in Tables 38 and 39, expressed as μg/g liver or kidney tissue. The kidney to liver ratio of full length oligonucleotide was also calculated and presented in Table 38.

Table 38

Full-length oligonucleotide concentration (μg/g) and ratio in the liver and kidney of Sprague-Dawley rats			
ISIS NO.	Liver	Kidney	Kidney/ Liver Ratio
304299	165	487	2.9
304309	344	606	1.8
420915	171	680	4.0
420951	214	389	1.8
425655	242	466	1.9
425656	286	595	2.1
425679	290	334	1.2
425695	266	566	2.1
425736	245	571	2.3
425737	167	477	2.9
425755	218	379	1.7

Table 39

Total oligonucleotide concentration (μg/g) in the liver and kidney of Sprague-Dawley rats		
ISIS NO.	Liver	Kidney
304299	208	653
304309	409	803
420915	196	844
420951	348	879
425655	340	764
425656	329	703
425679	461	710
425695	369	843
425736	282	738
425737	195	587
425755	351	886

Example 15: In vivo dose-dependent inhibition of human transthyretin in transgenic mice

[0393] Transgenic mice containing the human transthyretin gene were dosed in increasing doses of ISIS oligonucleotides selected from studies described in Example 14 to evaluate the effect of dose-dependent inhibition of human transthyretin in these mice.

Treatment

[0394] Groups of four mice, two male and two female, each were injected subcutaneously twice a week for 4 weeks with 4 mg/kg, 10 mg/kg or 25 mg/kg of ISIS 304299, ISIS 420915, ISIS 420951, ISIS 425679, ISIS 425736, ISIS 425737, or ISIS 425755. One group of four mice, two male and two female, was injected subcutaneously twice a week for 4 weeks with 25 mg/kg of the control oligonucleotide, ISIS 141923. One control group of four mice, two male and two female, was injected subcutaneously twice a week for 4 weeks with PBS. Plasma samples were taken from each group at days 0, 7, 14, 21 and 28. Two days after the last dose, the mice were euthanized and organs were harvested for further analysis.

RNA analysis

[0395] RNA was extracted from liver tissue for real-time PCR analysis of transthyretin using primer probe set RTS3029. Results are presented as percent inhibition of human transthyretin, relative to PBS control. As shown in Table 40, treatment with ISIS antisense oligonucleotides resulted in significant dose-dependent reduction of human transthyretin mRNA in comparison to the PBS control. Treatment with the control oligonucleotide, ISIS 141923 did not result in significant reduction of transthyretin, as expected.

Table 40

Inhibition of human transthyretin mRNA in the hTTR transgenic mice liver relative to the PBS control		
ISIS NO.	Dose (mg/kg)	% inhibition
304299	25	73
	10	60
	4	9
420915	25	78
	10	57
	4	43
420951	25	91
	10	85
	4	52
425679	25	94
	10	88
	4	42
425736	25	49
	10	54
	4	15
425737	25	82
	10	59
	4	21

(continued)

Inhibition of human transthyretin mRNA in the hTTR transgenic mice liver relative to the PBS control		
ISIS NO.	Dose (mg/kg)	% inhibition
425755	25	91
	10	79
	4	24
141923	25	0

Protein analysis

[0396] Human transthyretin protein levels were measured in transgenic mice plasma by ELISA using an anti-transthyretin polyclonal antibody (Abcam Ab37774) and a sheep anti-TTR horse radish peroxidase detection antibody (Abcam cat. no. 35217). The color reaction was developed by the ImmunoPure® TMB Substrate Kit and absorbance measured at 450 nm using a microtiter plate spectrophotometer. Plasma samples were taken predose and on days 7, 14,21 and 28. The results are presented in Table 41 expressed as percentage inhibition compared to the predose levels and demonstrate a time-dependent and dose-dependent reduction in protein levels on treatment with ISIS oligonucleotides.

Table 41

Inhibition of human transthyretin protein in transgenic mice plasma relative to pre-dose levels						
ISIS NO.		Day 0	Day 7	Day 14	Day 21	Day 28
141923	25	0	0	20	77	41
304299	25	0	44	85	100	88
	10	0	0	8	93	78
	4	0	0	0	57	0
420915	25	0	0	67	86	91
	10	0	21	39	70	71
	4	0	25	0	0	0
420951	25	0	83	96	100	100
	10	0	35	66	91	86
	4	0	7	26	0	0
425679	25	0	93	97	96	98
	10	0	38	80	96	95
	4	0	0	0	0	0
425736	25	0	56	76	82	92
	10	0	0	33	37	66
	4	0	0	0	0	0
425737	25	0	90	96	99	98
	10	0	51	80	88	89
	4	0	29	21	37	31
425755	25	0	88	96	98	99
	10	0	52	76	90	88
	4	0	29	22	36	26

Body weight and organ weight

[0397] The body weights of the mice were measured pre-dose and at the end of the treatment period. The body weights are presented in Table 42 and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 42 as a percentage change over the respective organ weights of the PBS control.

Table 42

Change in body and organ weights of transgenic mice after antisense oligonucleotide treatment (%)					
	Dose (mg/kg)	Body weight	Liver	Spleen	Kidney
PBS		+13	0	0	0
ISIS 304299	25	+17	+16	+3	-2
	10	+14	+10	-13	-4
	4	+17	+2	+17	-2
ISIS 420915	25	+18	+12	-6	-6
	10	+16	+6	-4	-5
	4	+15	+4	+8	-2
ISIS 420951	25	+22	+23	+32	-2
	10	+16	+11	+10	-3
	4	+24	+7	+19	+5
ISIS 425679	25	+24	+33	+40	-1
	10	+14	+5	+9	-2
	4	+19	+7	+10	0
ISIS 425736	25	+16	+15	0	-5
	10	+28	+8	-12	-6
	4	+20	+10	-9	-2
ISIS 425737	25	+16	+13	0	-2
	10	+19	+6	+18	-3
	4	+19	+5	+4	+1
ISIS 425755	25	+21	+25	+34	-5
	10	+17	+10	+13	-4
	4	+22	+3	+27	+4
ISIS 141923	25	+20	+8	-3	-4

Liver function

[0398] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 43 expressed in IU/L. Plasma levels of bilirubin were also measured using the same clinical chemistry analyzer; results are also presented in Table 43 and expressed in mg/dL.

Table 43

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of transgenic mice				
	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)	TBIL (mg/dL)
PBS	-	48	112	0.20
ISIS 304299	25	42	93	0.14
	10	37	56	0.18
	4	35	71	0.15
ISIS 420915	25	63	181	0.22
	10	46	132	0.22
	4	35	114	0.22
ISIS 420951	25	63	85	0.17
	10	42	107	0.21
	4	31	74	0.19
ISIS 425679	25	156	150	0.13
	10	93	148	0.23
	4	38	119	0.22
ISIS 425736	25	37	78	0.21
	10	33	62	0.20
	4	46	228	0.23
ISIS 425737	25	55	121	0.20
	10	41	94	0.18
	4	32	73	0.14
ISIS 425755	25	74	160	0.17
	10	31	80	0.16
	4	45	122	0.21
ISIS 141923	25	66	141	0.17

Kidney function

[0399] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 44, expressed in mg/dL.

Table 44

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of transgenic mice		
	Dose (mg/kg)	BUN
PBS		22
ISIS 304299	25	22
	10	22
	4	22

(continued)

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of transgenic mice		
	Dose (mg/kg)	BUN
ISIS 420915	25	24
	10	25
	4	20
ISIS 420951	25	24
	10	25
	4	26
ISIS 425679	25	26
	10	24
	4	22
ISIS 425736	25	20
	10	22
	4	22
ISIS 425737	25	21
	10	19
	4	23
ISIS 425755	25	23
	10	21
	4	20
ISIS 141923	25	21

Example 16: In vivo inhibition of human transthyretin in human transthyretin-transgenic mice

[0400] Antisense oligonucleotides with 5-10-5 MOE motifs, ISIS 304313, ISIS 420913, ISIS 420919, ISIS 420921, ISIS 420922, ISIS 420937, ISIS 420944, ISIS 420947, ISIS 420949, ISIS 420950, ISIS 420951, ISIS 420952, ISIS 420953, ISIS 420955, ISIS 420957, and ISIS 420959 from Table 4. These antisense oligonucleotides exhibited 65% inhibition or more of transthyretin mRNA were selected and tested in transgenic mice containing the human transthyretin gene. Additional oligonucleotides with overlapping sequences to ISIS 420951 (GTTTTATTGTCTCTGCCTGG (SEQ ID NO: 116)), and with various motifs were also designed to test in the transgenic mice. These additional oligonucleotides were ISIS 450518 (TTTTATTGTCTCTGCCTG (SEQ ID NO: 5-8-5 MOE (SEQ ID NO: 167)), ISIS 450519 (GTTTTATTGTCTCTGCCTGG, 6-8-6 MOE (SEQ ID NO: 116)), ISIS 450520 (GTTTTATTGTCTCTGCCTGG, 3-10-7 MOE (SEQ ID NO: 116)), ISIS 450521 (GTTTTATTGTCTCTGCCTGG, 7-10-3 MOE (SEQ ID NO: 116)), ISIS 450522 (GTTTTATTGTCTCTGCCTGG, 2-10-8 MOE (SEQ ID NO: 116)), and ISIS 450523 (GTTTTATTGTCTCTGCCTGG, 8-10-2 MOE (SEQ ID NO: 116)).

Treatment

[0401] Groups of four hTTR transgenic mice each, two male and two female, were administered subcutaneously twice per week for four weeks with 25 mg/kg of ISIS 304313, ISIS 420913, ISIS 420919, ISIS 420921, ISIS 420922, ISIS 420937, ISIS 420944, ISIS 420947, ISIS 420949, ISIS 420950, ISIS 420951, ISIS 420952, ISIS 420953, ISIS 420955, ISIS 420957, ISIS 420959, ISIS 425518, ISIS 425519, ISIS 425520, ISIS 425521, ISIS 425522, or ISIS 425523. A control group four hTTR transgenic mice, two male and two female, were injected subcutaneously with PBS twice per week for four weeks. Blood samples were collected from all groups on days 0, 14 and 28 for plasma transthyretin level analysis. The mice were sacrificed two days after the last dose and livers were harvested for target mRNA analysis.

RNA analysis

[0402] RNA was extracted from liver tissue for real-time PCR analysis of transthyretin using primer probe set RTS3029. Results are presented as percent inhibition of human transthyretin, relative to PBS control. As shown in Table 45, treatment with ISIS antisense oligonucleotides resulted in significant reduction of human transthyretin mRNA in comparison to the PBS control.

Table 45

Inhibition of human transthyretin mRNA in the hTTR transgenic mice liver relative to the PBS control	
ISIS NO.	% inhibition
304313	68
420913	83
420919	64
420921	70
420922	82
420937	46
420944	58
420947	62
420949	87
420950	94
420952	95
420953	93
420955	93
420957	90
420959	73
450518	80
450519	87
450520	85
450521	94
450522	73
450523	94
420951	94

Protein analysis

[0403] Human transthyretin protein levels were measured in transgenic mice plasma by ELISA using an anti-transthyretin transthyretin polyclonal antibody (Abcam Ab37774) and a sheep anti-TTR horse radish peroxidase detection antibody (Abcam cat. no. 35217). The color reaction was developed by the ImmunoPure® TMB Substrate Kit and absorbance measured at 450 nm using a microtiter plate spectrophotometer. Plasma samples were taken predose and on days 7, 14 and 28. The results are presented in Table 46 expressed as percentage inhibition compared to the pre-dose levels and demonstrate a time-dependent reduction in protein levels on treatment with ISIS oligonucleotides.

Table 46

Inhibition of human transthyretin protein in the hTTR transgenic mice plasma relative to pre-dose levels			
	Day 0	Day 14	Day 28
PBS	0	0	0
ISIS 304313	0	62	77
ISIS 420913	0	91	97
ISIS 420919	0	70	82
ISIS 420921	0	83	87
ISIS 420922	0	95	97
ISIS 420937	0	37	59
ISIS 420944	0	57	72
ISIS 420947	0	57	65
ISIS 420949	0	93	99
ISIS 420950	0	97	100
ISIS 420952	0	98	100
ISIS 420953	0	99	100
ISIS 420955	0	89	100
ISIS 420957	0	92	94
ISIS 420959	0	69	87
ISIS 450518	0	80	97
ISIS 450519	0	94	100
ISIS 450520	0	83	100
ISIS 450521	0	100	100
ISIS 450522	0	93	97
ISIS 450523	0	100	100
ISIS 420951	0	99	100

Liver function

[0404] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 47 expressed in IU/L. Plasma levels of bilirubin were also measured using the same clinical chemistry analyzer; results are also presented in Table 47 and expressed in mg/dL.

Table 47

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of transgenic mice			
	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)
PBS	34	88	0.20
ISIS 304313	42	79	0.16
ISIS 420913	35	67	0.17
ISIS 420919	63	177	0.20
ISIS 420921	47	103	0.15

(continued)

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of transgenic mice			
	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)
ISIS 420922	42	128	0.16
ISIS 420937	33	160	0.15
ISIS 420944	38	84	0.15
ISIS 420947	42	120	0.17
ISIS 420949	46	125	0.15
ISIS 420950	73	106	0.15
ISIS 420952	151	271	0.19
ISIS 420953	982	452	0.16
ISIS 420955	47	80	0.15
ISIS 420957	53	133	0.18
ISIS 420959	31	89	0.11
ISIS 450518	103	200	0.20
ISIS 450519	64	81	0.12
ISIS 450520	350	270	0.12
ISIS 450521	104	226	0.13
ISIS 450522	109	201	0.14
ISIS 450523	80	170	0.19
ISIS 420951	67	100	0.09

Kidney function

[0405] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 48, expressed in mg/dL.

Table 48

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of transgenic mice	
PBS	35
ISIS 304313	29
ISIS 420913	30
ISIS 420919	29
ISIS 420921	29
ISIS 420922	27
ISIS 420937	29
ISIS 420944	27
ISIS 420947	26
ISIS 420949	25
ISIS 420950	34
ISIS 420952	23

(continued)

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of transgenic mice	
PBS	35
ISIS 420953	34
ISIS 420955	24
ISIS 420957	23
ISIS 420959	29
ISIS 450518	28
ISIS 450519	25
ISIS 450520	29
ISIS 450521	24
ISIS 450522	29
ISIS 450523	27
ISIS 420951	25

Example 17: Tolerability of antisense oligonucleotides targeting human transthyretin in CD1 mice

[0406] CD1 mice were treated with ISIS antisense oligonucleotides from Example 16 and evaluated for changes in the levels of various metabolic markers.

Treatment

[0407] Groups of eight CD1 mice each were injected subcutaneously twice a week with 50 mg/kg of ISIS 304313, ISIS 420913, ISIS 420919, ISIS 420921, ISIS 420922, ISIS 420937, ISIS 420944, ISIS 420947, ISIS 420949, ISIS 420950, ISIS 420951, ISIS 420952, ISIS 420953, ISIS 420955, ISIS 420957, ISIS 420959, ISIS 425518, ISIS 425519, ISIS 425520, ISIS 425521, ISIS 425522, or ISIS 425523. Three days after the last dose at each time point, body weights were taken, mice were euthanized and organs and plasma were harvested for further analysis.

Body and organ weights

[0408] The body weights of the mice were measured pre-dose and at the end of each treatment period (two weeks and six weeks). The body weights are presented in Table 49 and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 49 as a percentage change over the respective organ weights of the PBS control.

Table 49

Change in body and organ weights of CD1 mice after antisense oligonucleotide treatment (%) at week 6				
	Body weight	Liver	Spleen	Kidney
PBS	1.3	1.0	1.0	1.0
ISIS 304313	1.2	1.2	1.4	1.2
ISIS 420913	1.2	1.2	1.3	1.1
ISIS 420919	1.3	1.2	1.9	1.1
ISIS 420921	1.1	1.1	2.2	1.1
ISIS 420922	1.1	1.0	1.6	0.9
ISIS 420937	1.1	1.0	1.2	1.0
ISIS 420944	1.1	1.1	2.0	1.0

(continued)

Change in body and organ weights of CD1 mice after antisense oligonucleotide treatment (%) at week 6				
	Body weight	Liver	Spleen	Kidney
ISIS 420947	1.3	1.2	1.7	1.0
ISIS 420949	1.3	1.2	1.8	1.1
ISIS 420950	1.3	1.0	1.7	1.0
ISIS 420952	1.4	1.3	2.1	0.9
ISIS 420953	1.3	1.5	2.2	1.0
ISIS 420955	1.2	1.2	2.2	1.0
ISIS 420957	1.1	1.1	1.8	1.1
ISIS 420959	1.3	1.2	3.2	1.1
ISIS 450518	1.4	1.3	1.8	1.1
ISIS 450519	1.3	1.5	2.4	1.0
ISIS 450520	1.4	1.4	2.2	1.0
ISIS 450521	1.2	1.2	1.9	1.1
ISIS 450522	1.3	1.5	2.3	1.1
ISIS 450523	1.2	1.3	2.4	1.1
ISIS 420951	1.3	1.2	1.9	1.0

Liver function

[0409] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 50 expressed in IU/L. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer and the results are also presented in Table 50.

Table 50

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of CD1 mice			
	ALT	AST	TBIL
PBS	34	88	0.20
ISIS 304313	42	79	0.16
ISIS 420913	35	67	0.17
ISIS 420919	63	177	0.20
ISIS 420921	47	103	0.15
ISIS 420922	42	128	0.16
ISIS 420937	33	160	0.15
ISIS 420944	38	84	0.15
ISIS 420947	42	120	0.17
ISIS 420949	46	125	0.15
ISIS 420950	73	106	0.15
ISIS 420952	151	271	0.19
ISIS 420953	982	452	0.16

(continued)

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of CD1 mice			
	ALT	AST	TBIL
ISIS 420955	47	80	0.15
ISIS 420957	53	133	0.18
ISIS 420959	31	89	0.11
ISIS 450518	103	200	0.20
ISIS 450519	64	81	0.12
ISIS 450520	350	270	0.12
ISIS 450521	104	226	0.13
ISIS 450522	109	201	0.14
ISIS 450523	80	170	0.19
ISIS 420951	67	100	0.09

Kidney function

[0410] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 51, expressed in mg/dL.

Table 51

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of CD1 mice	
	BUN
PBS	35
ISIS 304313	29
ISIS 420913	30
ISIS 420919	29
ISIS 420921	29
ISIS 420922	27
ISIS 420937	29
ISIS 420944	27
ISIS 420947	26
ISIS 420949	25
ISIS 420950	34
ISIS 420952	23
ISIS 420953	34
ISIS 420955	24
ISIS 420957	23
ISIS 420959	29
ISIS 450518	28
ISIS 450519	25
ISIS 450520	29

(continued)

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of CD1 mice	
	BUN
ISIS 450521	24
ISIS 450522	29
ISIS 450523	27
ISIS 420951	25

Hematology assays

[0411] Blood obtained from all mice groups were sent to Antech Diagnostics for hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) measurements and analyses, as well as measurements of the differential blood cell counts, such as that of WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Table 52 and 53. Percentages given in the tables indicate the percent change in total blood cell count compared to the PBS control.

Table 52

Effect of antisense oligonucleotide treatment on complete blood cell count (%) compared to the PBS control in CD1 mice							
	WBC	RBC	Hemoglobin	HCT	MCV	MCH	MCHC
ISIS 304313	+80	-5	-7	-9	-4	-2	+4
ISIS 420913	-10	-1	-3	-5	-4	-2	+3
ISIS 420919	+26	-2	-7	-9	-7	-5	+4
ISIS 420921	+60	-9	-12	-15	-6	-3	+5
ISIS 420922	+18	-6	-11	-16	-11	-6	+6
ISIS 420937	+42	-3	-4	-7	-5	-1	+5
ISIS 420944	+49	-5	-9	-13	-8	-4	+6
ISIS 420947	+36	-2	-2	-5	-3	0	+4
ISIS 420949	+61	-4	-6	-9	-7	-3	+5
ISIS 420950	+56	-14	-16	-19	-7	-3	+6
ISIS 420952	+36	-20	-24	-25	-7	-5	+4
ISIS 420953	+105	-21	-24	-26	-6	-4	+4
ISIS 420955	+107	-14	-19	-21	-9	-5	+6
ISIS 420957	+79	-5	-10	-13	-9	-6	+5
ISIS 420959	+92	-8	-14	-18	-11	-7	+6
ISIS 450518	+138	-5	-10	-12	-7	-4	+4
ISIS 450519	+118	-17	-21	-24	-9	-5	+6
ISIS 450520	+151	-18	-21	-23	-7	-4	+4
ISIS 450521	+118	-15	-21	-23	-11	-7	+5
ISIS 450522	+63	-22	-28	-31	-12	-8	+6
ISIS 450523	+116	-22	-27	-29	-11	-7	+6
ISIS 420951	+54	-15	-21	-24	-10	-6	+5

Table 53

Effect of antisense oligonucleotide treatment on differential blood cell count (%) compared to the PBS control in CD1 mice				
	Neutrophils	Lymphocytes	Monocytes	Platelets
ISIS 304313	-54	+49	-45	+36
ISIS 420913	-46	+39	-21	-2
ISIS 420919	-57	+49	-21	+19
ISIS 420921	-55	+47	-24	+25
ISIS 420922	-53	+46	-31	+24
ISIS 420937	-63	+57	-48	+20
ISIS 420944	-40	+37	-28	+18
ISIS 420947	-55	+49	-38	-9
ISIS 420949	-30	+24	+7	+17
ISIS 420950	-50	+40	0	+6
ISIS 420952	-34	+33	-28	+13
ISIS 420953	-37	+35	-34	+11
ISIS 420955	-37	+34	-21	+30
ISIS 420957	-71	+61	-28	+16
ISIS 420959	-52	+45	-24	-1
ISIS 450518	-56	+49	-28	+18
ISIS 450519	-18	+11	+41	+55
ISIS 450520	-41	+34	0	+7
ISIS 450521	-41	+36	-14	+21
ISIS 450522	-41	+31	+17	+58
ISIS 450523	-28	+19	+31	+51
ISIS 420951	-28	+24	0	+26

Example 18: Tolerability of antisense oligonucleotides targeting human transthyretin in Sprague-Dawley rats

[0412] ISIS oligonucleotides selected from studies described in Example 17 were also tested in Sprague-Dawley rats and evaluated for changes in the levels of various metabolic markers.

Treatment

[0413] The body weights, complete blood count and different blood count, as well as the urine protein/ creatinine ratio of the rats were evaluated pre-dose. Groups of four Sprague-Dawley rats each were injected subcutaneously twice a week with 50 mg/kg of ISIS 420913, ISIS 420921, ISIS 420922, ISIS 420950, ISIS 420955, ISIS 420957, and ISIS 420959. Three days after the last dose at each time point, body weights were taken, mice were euthanized and organs and plasma were harvested for further analysis.

Body and organ weights

[0414] The body weights of the rats were measured pre-dose and at the end of the treatment period. The body weights are presented in Table 54, and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 54 as a percentage change over the respective organ weights of the PBS control.

Table 54

Change in body and organ weights of Sprague-Dawley rats after antisense oligonucleotide treatment (%)				
	Body weight	Liver	Spleen	Kidney
PBS	2.1	1.0	1.0	1.0
ISIS 420913	1.5	1.5	4.7	1.1
ISIS 420921	1.6	1.5	4.2	1.3
ISIS 420922	1.3	1.5	4.4	1.4
ISIS 420950	1.4	1.5	6.4	1.7
ISIS 420955	1.5	1.6	5.9	1.4
ISIS 420957	1.4	1.4	6.8	1.3
ISIS 420959	1.5	1.4	5.5	1.4

[0415] As shown in Table 54, the compounds demonstrated a less than 10-fold increase in organ weight of these rats. Furthermore, certain compounds demonstrated a less than 7-fold increase in organ weight of these rats. While certain compounds demonstrated a less than 6-fold increase in organ weight of these rats. Certain compounds demonstrated a less than 5-fold increase in organ weight of these rats.

Liver function

[0416] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 55 expressed in IU/L. Plasma levels of bilirubin and albumin were also measured using the same clinical chemistry analyzer and the results are also presented in Table 55.

Table 55

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of Sprague-Dawley rats				
	ALT (IU/L	AST (IU/L)	TBIL (mg/dL)	Albumin (g/dL)
PBS	26	66	0.09	4.5
ISIS 420913	38	95	0.08	3.3
ISIS 420921	65	151	0.11	3.2
ISIS 420922	40	121	0.11	4.0
ISIS 420950	398	327	0.19	4.0
ISIS 420955	78	241	0.18	4.1
ISIS 420957	84	244	0.14	3.7
ISIS 420959	82	405	0.17	4.6

Kidney function

[0417] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 56, expressed in mg/dL. The ratio of total urine protein to creatinine was also evaluated and presented in Table 56.

Table 56

Effect of antisense oligonucleotide treatment on metabolic markers (mg/dL) in the kidney of Sprague-Dawley rats		
	BUN	Creatinine
PBS	14	0.05
ISIS 420913	22	0.09
ISIS 420921	23	0.07
ISIS 420922	21	0.08
ISIS 420950	20	0.11
ISIS 420955	22	0.06
ISIS 420957	23	0.18
ISIS 420959	24	0.17

Table 57

Effect of antisense oligonucleotide treatment on total urine protein/ creatinine in the kidney of Sprague-Dawley rats	
	Urine protein/creatinine ratio
PBS	1.50
ISIS 420913	19.51
ISIS 420921	5.07
ISIS 420922	4.72
ISIS 420950	5.61
ISIS 420955	5.57
ISIS 420957	5.40
ISIS 420959	4.39

Hematology assays

[0418] Blood obtained from all rat groups were sent to Antech Diagnostics for hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) measurements and analyses, as well as measurements of the differential blood cell counts, such as that of WBC (neutrophils, lymphocytes, and monocytes), RBC, and platelets, and total hemoglobin content. The results are presented in Tables 58 and 59. Percents given in the tables indicate the percent change in total blood cell count compared to the PBS control.

Table 58

Effect of antisense oligonucleotide treatment on complete blood cell count (%) compared to the PBS control in Sprague-Dawley rats							
	WBC	RBC	Hemoglobin	HCT	MCV	MCH	MCHC
PBS	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ISIS 420913	1.7	0.9	0.9	0.9	0.9	0.9	1.0
ISIS 420921	1.6	0.9	0.9	0.9	1.0	1.0	1.0
ISIS 420922	1.6	0.9	0.9	0.8	1.0	1.0	1.0
ISIS 420950	2.2	0.7	0.7	0.7	1.0	1.0	1.0
ISIS 420955	1.9	0.7	0.8	0.7	1.1	1.2	1.0
ISIS 420957	3.1	0.8	0.8	0.8	1.0	1.0	1.0

(continued)

Effect of antisense oligonucleotide treatment on complete blood cell count (%) compared to the PBS control in Sprague-Dawley rats							
	WBC	RBC	Hemoglobin	HCT	MCV	MCH	MCHC
ISIS 420959	2.2	0.8	0.8	0.8	1.0	1.0	1.0

Table 59

Effect of antisense oligonucleotide treatment on differential blood cell count (%) compared to the PBS control in Sprague-Dawley rats				
	Neutrophils	Lymphocytes	Monocytes	Platelet
PBS	1.0	1.0	1.0	1.0
ISIS 420913	0.5	1.1	1.7	0.7
ISIS 420921	0.7	1.0	1.6	0.6
ISIS 420922	0.5	1.1	1.3	0.7
ISIS 420950	0.8	1.0	2.3	0.7
ISIS 420955	0.5	1.0	2.4	0.7
ISIS 420957	0.7	1.0	1.6	0.3
ISIS 420959	0.5	1.1	1.3	n.d.

Example 19: Pharmacokinetic studies of half-life of antisense oligonucleotide concentration in Sprague-Dawley rat liver and kidney

[0419] Sprague Dawley rats were treated with ISIS antisense oligonucleotides targeting from studies described in Example 18 and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver and kidney was evaluated.

Treatment

[0420] Groups of four Sprague Dawley rats each were injected subcutaneously twice a week for 2 weeks with 20 mg/kg of ISIS 420913, ISIS 420921, ISIS 420922, ISIS 420950, ISIS 420955, ISIS 420957, and ISIS 420959. Three days after the last dose, the rats were sacrificed and livers and kidneys were collected for analysis.

Measurement of oligonucleotide concentration

[0421] The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenolchloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTT-GCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 166) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. The results are presented in Tables 60 and 61, expressed as μg/g liver or kidney tissue. The kidney to liver ratio of oligonucleotide concentration was also calculated and presented in Tables 60 and 61.

Table 60

Full-length oligonucleotide concentration (μg/g) and ratio in the liver and kidney of Sprague-Dawley rats			
ISIS NO.	Liver	Kidney	Kidney/Liver ratio
420913	154	285	1.9
420921	147	293	2.0

(continued)

Full-length oligonucleotide concentration (μg/g) and ratio in the liver and kidney of Sprague-Dawley rats			
ISIS NO.	Liver	Kidney	Kidney/Liver ratio
420922	226	497	2.2
420950	161	411	2.6
420955	152	383	2.5
420957	235	453	1.9
420959	187	513	2.7

Table 61

Total oligonucleotide concentration (μg/g) in the liver and kidney of Sprague-Dawley rats			
ISIS NO.	Liver	Kidney	Kidney/Liver ratio
420913	180	310	1.7
420921	159	305	1.9
420922	238	544	2.3
420950	168	466	2.8
420955	156	442	2.8
420957	244	551	2.3
420959	202	534	2.6

Example 20: In vivo dose-dependent inhibition of human transthyretin in transgenic mice

[0422] ISIS 420913, ISIS 420921, ISIS 420922, ISIS 420957 and ISIS 420959, which exhibited good efficacy and tolerability, as demonstrated in Examples 16-19, were chosen for the study of dose-dependent target knockdown in transgenic mice containing the human transthyretin gene. ISIS 420950 and ISIS 420955, which demonstrated 90% or more target knockdown, but which also demonstrated toxicity in CD1 mice (Examples 16-19) were also chosen for this study for comparison.

Treatment

[0423] Groups of four mice, two male and two female, each were injected subcutaneously twice a week for 4 weeks with 4 mg/kg, 10 mg/kg or 25 mg/kg of ISIS 420913, ISIS 420921, ISIS 420922, ISIS 420950, ISIS 420955, ISIS 420957, or ISIS 420959. One group of four mice, two male and two female, was injected subcutaneously twice a week for 4 weeks with 25 mg/kg of the control oligonucleotide, ISIS 141923. One control group of four mice, two male and two female, was injected subcutaneously twice a week for 4 weeks with PBS. Plasma samples were taken from each group at days 0, 14 and 28. Two days after the last dose, the mice were euthanized and organs were harvested for further analysis.

RNA analysis

[0424] RNA was extracted from liver tissue for real-time PCR analysis of transthyretin using primer probe set RTS3029. Results are presented as percent inhibition of human transthyretin, relative to PBS control. As shown in Table 62, treatment with ISIS antisense oligonucleotides resulted in significant dose-dependent reduction of human transthyretin mRNA in comparison to the PBS control. Treatment with the control oligonucleotide, ISIS 141923 did not result in significant reduction of transthyretin, as expected.

Table 62

Inhibition of human transthyretin mRNA in the hTTR transgenic mice liver relative to the PBS control		
ISIS NO.	Dose (mg/kg)	% inhibition
420913	25	78
	10	65
	4	32
420921	25	76
	10	64
	4	13
420922	25	80
	10	53
	4	21
420950	25	92
	10	77
	4	57
420955	25	88
	10	56
	4	23
420957	25	85
	10	72
	4	32
420959	25	75
	10	26
	4	11
141923	25	0

Protein analysis

[0425] Human transthyretin protein levels were measured in transgenic mice plasma by ELISA using an anti-transthyretin transthyretin polyclonal antibody (Abcam Ab37774) and a sheep anti-TTR horse radish peroxidase detection antibody (Abcam cat. no. 35217). The color reaction was developed by the ImmunoPure® TMB Substrate Kit and absorbance measured at 450 nm using a microtiter plate spectrophotometer. Plasma samples were taken predose and on days 7, 14, 21 and 28. The results are presented in Table 63 expressed as percentage inhibition compared to the predose levels and demonstrate a time-dependent and dose-dependent reduction in protein levels on treatment with ISIS oligonucleotides.

Table 63

Inhibition of human transthyretin protein in the hTTR transgenic mice plasma relative to predose levels				
ISIS NO.	Dose (mg/kg)	d0	d14	d28
420913	25	0	73	93
	10	0	27	96
	4	0	25	54

(continued)

Inhibition of human transthyretin protein in the hTTR transgenic mice plasma relative to predose levels				
ISIS NO.	Dose (mg/kg)	d0	d14	d28
420921	25	0	73	90
	10	0	63	79
	4	0	42	67
420922	25	0	63	96
	10	0	57	89
	4	0	38	77
420950	25	0	95	97
	10	0	71	96
	4	0	29	53
420955	25	0	84	96
	10	0	53	91
	4	0	20	30
420957	25	0	83	93
	10	0	51	66
	4	0	32	49
420959	25	0	74	80
	10	0	31	58
	4	0	0	0
141923	25	0	22	0

Body weight and organ weight

[0426] The body weights of the mice were measured pre-dose and at the end of the treatment period. The body weights are presented in Table 64 and are expressed as percent increase over the PBS control weight taken before the start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 64 as a percentage change over the respective organ weights of the PBS control.

Table 64

Change in body and organ weights of transgenic mice after antisense oligonucleotide treatment (%)					
		Body weight	Liver	Spleen	Kidney
PBS		6.4	0.0	0.0	0.0
ISIS 420913	25	8.1	0.3	11.4	4.1
	10	10.6	-8.6	14.3	13.6
	4	7.4	3.7	5.0	12.0
ISIS 420921	25	10.5	8.8	25.6	-0.1
	10	9.7	5.7	10.8	4.0
	4	8.7	-4.4	16.0	11.0

(continued)

Change in body and organ weights of transgenic mice after antisense oligonucleotide treatment (%)					
		Body weight	Liver	Spleen	Kidney
ISIS 420922	25	8.4	5.6	18.0	1.7
	10	9.2	-1.7	27.1	6.3
	4	8.1	-2.1	-11.4	5.1
ISIS 420950	25	12.8	14.3	22.8	1.7
	10	8.4	4.3	-2.8	0.6
	4	9.1	0.4	14.2	1.5
ISIS 420955	25	10.1	14.6	17.7	-4.4
	10	11.8	5.6	-0.3	1.4
	4	7.9	4.7	-12.3	4.5
ISIS 420957	25	12.8	6.4	33.1	2.8
	10	14.5	13.9	-6.3	9.7
	4	7.4	-5.4	12.2	6.2
ISIS 420959	25	10.0	2.4	72.7	23.3
	10	7.2	-5.4	40.2	9.8
	4	4.1	-4.4	27.8	-6.6
ISIS 141923	25	9.2	-1.3	20.4	-5.5

Liver function

[0427] To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 65 expressed in IU/L. Plasma levels of bilirubin were also measured using the same clinical chemistry analyzer; results are also presented in Table 65 and expressed in mg/dL.

Table 65

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of transgenic mice				
	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)	TBIL (mg/dL)
PBS		47	63	0.16
ISIS 420913	25	42	69	0.13
	10	49	90	0.17
	4	42	59	0.18
ISIS 420921	25	56	96	0.12
	10	51	68	0.22
	4	42	75	0.14
ISIS 420922	25	50	76	0.12
	10	40	170	0.14
	4.	37	48	0.13

(continued)

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of transgenic mice				
	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)	TBIL (mg/dL)
ISIS 420950	25	74	116	0.14
	10	37	67	0.13
	4	34	64	0.11
ISIS 420955	25	46	117	0.15
	10	54	76	0.16
	4	50	153	0.17
ISIS 420957	25	40	73	0.13
	10	36	63	0.20
	4	37	61	0.12
ISIS 420959	25	51	92	0.19
	10	48	69	0.13
	4	37	67	0.13
ISIS 141923	25	44	79	0.12

Kidney function

[0428] To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 66, expressed in mg/dL.

Table 66

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of transgenic mice		
	Dose (mg/kg)	BUN
PBS	-	23
ISIS 420913	25	24
	10	22
	4	20
ISIS 420921	25	24
	10	22
	4	23
ISIS 420922	25	23
	10	22
	4	24
ISIS 420950	25	22
	10	26
	4	23
ISIS 420955	25	23
	10	24
	4	25

(continued)

Effect of antisense oligonucleotide treatment on BUN (mg/dL) in the kidney of transgenic mice		
	Dose (mg/kg)	BUN
ISIS 420957	25	20
	10	22
	4	20
ISIS 420959	25	25
	10	22
	4	22
ISIS 141923	25	19

Example 21: Dose response confirmation of antisense oligonucleotides targeting human transthyretin in cynomolgus monkey primary hepatocytes

[0429] Gapmers showing tolerability in CD1 mice and Sprague Dawley rats (studies described in Examples 17-19) as well as potency in transgenic mice (studies described in Examples 16 and 20) were selected and tested at various doses in primary hepatocytes of cynomolgus monkeys. Cells were plated at a density of 35,000 cells per well and transfected using electroporation with 156.25 nM, 312.5 nM, 625 nM, 1,250 nM 2,500 nM, 5,000 nM, 10,000 nM and 20,000 nM concentrations of antisense oligonucleotide, as specified in Table 67. After a treatment period of approximately 16 hours, RNA was isolated from the cells and transthyretin mRNA levels were measured by quantitative real-time PCR. Human transthyretin primer probe set RTS1396 was used to measure mRNA levels. Transthyretin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of transthyretin, relative to untreated control cells. As illustrated in Table 67, transthyretin mRNA levels were reduced in a dose-dependent manner in hepatocytes treated with all the ISIS oligonucleotides, which are cross-reactive with rhesus monkey transthyretin gene, designated herein as SEQ ID NO: 4 (exons 1-4 extracted from GENBANK Accession No. NW_001105671.1).

Table 67

Dose-dependent antisense inhibition of human transthyretin in cynomolgus monkey primary hepatocytes using electroporation										
ISIS No.	156.25 nM	312.5 nM	625 nM	1250 nM	2500 nM	5000 nM	10000 nM	20000 nM	IC_{50} (μM)	Target Start Site
304299	0	0	25	42	89	95	98	99	1.4	504
420913	0	0	42	49	84	96	98	98	1.2	502
420915	0	8	46	58	84	94	97	99	1	505
420921	0	0	26	30	53	74	94	97	2	512
420922	4	0	13	29	38	69	87	97	2.9	513
420950	23	27	60	71	88	94	98	98	0.6	577
420955	19	0	25	50	74	86	93	97	1.4	582
420957	0	0	15	34	65	72	87	94	2.2	584
420959	3	12	10	37	71	88	94	94	1.5	586

Example 22: Measurement of viscosity of ISIS antisense oligonucleotides targeting human transthyretin

[0430] The viscosity of antisense oligonucleotides from studies described in Example 21 was measured with the aim of screening out antisense oligonucleotides which have a viscosity more than 40 cP. Oligonucleotides having a viscosity greater than 40 cP would be too viscous to be administered to any subject.

[0431] ISIS oligonucleotides (32-35 mg) were weighed into a glass vial, 120 μL of water was added and the antisense oligonucleotide was dissolved into solution by heating the vial at 50°C. Part of (75 μL) the pre-heated sample was pipetted to a micro-viscometer (Cambridge). The temperature of the micro-viscometter was set to 25°C and the viscosity of the sample was measured. Another part (20 μL) of the pre-heated sample was pipetted into 10 mL of water for UV reading at 260 nM at 85°C (Cary UV instrument). The results are presented in Table 68 and indicate that all the antisense oligonucleotides solutions are optimal in their viscosity under the criterion stated above.

Table 68

Viscosity and concentration of ISIS antisense oligonucleotides targeting human transthyretin		
ISIS No.	Viscosity (cP)	Concentration (mg/mL)
304299	9.9	169
420913	6.5	178
420915	8.4	227
420921	8.2	234
420922	5.3	191
420950	12.5	297
420955	15.7	259
420957	12.9	233
420959	18.7	276

Example 23: Measurement of half-life of antisense oligonucleotide in CD1 mouse liver

[0432] CD 1 mice were treated with ISIS antisense oligonucleotides from studies described in Example 22 and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver was evaluated.

Treatment

[0433] Groups of twelve CD1 mice each were injected subcutaneously twice per week for 2 weeks with 50 mg/kg of ISIS 420913, ISIS 420921, ISIS 420922, ISIS 420950, ISIS 420955, ISIS 420957, and ISIS 420959. Four mice from each group were sacrificed 3 days, 28 days and 56 days following the final dose. Livers were harvested for analysis.

Measurement of oligonucleotide concentration

[0434] The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 166) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. Half-lives were then calculated using WinNonlin software (PHARSIGHT).

[0435] The results are presented in Tables 69, expressed as μg/g liver tissue. The half-life of each oligonucleotide is presented in Table 70.

Table 69

Full-length oligonucleotide concentration (μg/g) in the liver of CD1 mice			
ISIS No.	3 days	28 days	56 days
420913	243	109	33
420921	225	49	6

(continued)

Full-length oligonucleotide concentration (μg/g) in the liver of CD1 mice			
ISIS No.	3 days	28 days	56 days
420922	310	129	53
420950	254	88	62
420955	308	137	79
420957	325	129	49
420959	258	97	37

Table 70

Half-life of oligonucleotide (days) in the liver of CD1 mice	
ISIS No.	Half-life (days)
420913	18.5
420921	10.0
420922	20.7
420950	26.4
420955	27.2
420957	19.5
420959	18.9

Example 24: Effect of ISIS antisense oligonucleotides targeting human transthyretin in cynomolgus monkeys

[0436] Cynomolgus monkeys were treated with ISIS antisense oligonucleotides from studies described in Examples 21, 22 and 23. Antisense oligonucleotide efficacy and tolerability, as well as their pharmacokinetic profile in the liver and kidney, were evaluated.

Treatment

[0437] Prior to the study, the monkeys were kept in quarantine for a 30-day time period, during which standard panels of serum chemistry and hematology, examination of fecal samples for ova and parasites, and a tuberculosis test, were conducted to screen out abnormal or ailing monkeys. Nine groups of four randomly assigned male cynomolgus monkeys each were injected subcutaneously thrice per week for the first week, and subsequently twice a week for the next 11 weeks, with 25 mg/kg of ISIS 304299, ISIS 420915, ISIS 420921, ISIS 420922, ISIS 420950, ISIS 420955, ISIS 420957, or ISIS 420959. A control group of 4 cynomolgus monkeys was injected with PBS subcutaneously thrice per week for the first week, and subsequently twice a week for the next 11 weeks. Blood samples were collected 5 days before the treatment as well as on various days of the study period and analyzed. The animals were fasted for at least 13 hours (overnight) prior to blood collection. Terminal sacrifices of all groups were conducted on day 86, which was 48 hours after the last dose.

[0438] During the study period, the monkeys were observed daily for signs of illness or distress. Any animal showing adverse effects to the treatment was removed and referred to the veterinarian and Study Director. All the animals treated with ISIS 420955 were removed from the study on day 31 due to symptoms of illness displayed by 2 monkeys in the group. Similarly, one monkey each from groups treated with ISIS 420957 and ISIS 420950 was removed from the study on days 44 and 76, respectively, due to signs of illness.

Inhibition studies

RNA analysis

[0439] On day 86, RNA was extracted from liver tissue for real-time PCR analysis of transthyretin using primer probe set RTS3029. Results are presented as percent inhibition of transthyretin, relative to PBS control, normalized to cyclophilin. Similar results were obtained on normalization with RIBOGREEN®. As shown in Table 71, treatment with ISIS antisense oligonucleotides resulted in significant reduction of transthyretin mRNA in comparison to the PBS control. Specifically, treatment with ISIS 420915 caused greater inhibition of TTR mRNA than treatment with ISIS 304299, even though the two oligonucleotides differ from each other by a single base-pair shift. The data for animals treated with ISIS 420955 was taken at day 31.

Table 71

Inhibition of transthyretin mRNA in the cynomolgus monkey liver relative to the PBS control	
ISIS No	**% inhibition**
304299	59
420915	78
420921	54
420922	61
420950	91
420955*	79
420957	64
420959	55
(*Data of day 31)	

Protein analysis

[0440] The monkeys were fasted overnight prior to blood collection. Approximately 1 mL of blood was collected from all available animals and placed in tubes containing the potassium salt of EDTA. The tubes were centrifuged (3000 rpm for 10 min at room temperature) to obtain plasma. Transthyretin protein levels were measured in the plasma using a clinical analyzer. Plasma samples were taken predose (on day -5) and on days 1, 9, 16, 23, 30, 44, 58, 72, and 86. The results are presented in Table 72 expressed as percentage inhibition compared to the predose levels and demonstrate a time-dependent reduction in protein levels with treatment with ISIS oligonucleotides. The final plasma TTR levels are presented in Table 73 and demonstrate the strong correlation between TTR protein level reduction and TTR mRNA inhibition (Table 71). Specifically, treatment with ISIS 420915 caused greater inhibition of TTR plasma protein than treatment with ISIS 304299 (76% inhibition vs. 47% inhibition), even though the two oligonucleotides differ from each other by a single base-pair shift.

Table 72

Time course of transthyretin protein level reduction in the cynomolgus monkey plasma relative to predose levels									
ISIS No.	**Day 0**	**Day 9**	**Day 16**	**Day 23**	**Day 30**	**Day 44**	**Day 58**	**Day 72**	**Day 86**
304299	4	15	21	23	26	27	31	38	47
420915	2	8	23	34	42	54	63	70	76
420921	5	11	21	31	23	27	30	40	50
420922	0	17	37	42	49	49	50	49	54
420950	0	39	63	68	72	79	85	82	87
420955	0	42	63	80	81	n/a	n/a	n/a	n/a
420957	2	18	28	26	26	35	35	41	50

(continued)

Time course of transthyretin protein level reduction in the cynomolgus monkey plasma relative to predose levels									
ISIS No.	**Day 0**	**Day 9**	**Day 16**	**Day 23**	**Day 30**	**Day 44**	**Day 58**	**Day 72**	**Day 86**
420959	0	25	29	28	32	38	42	43	50
n/a= study was terminated on day 31 for animals treated with ISIS 420955; therefore data for subsequent days is not available.									

Table 73

Day 86 transthyretin protein level reduction in the cynomolgus monkey plasma relative to predose levels	
ISIS No.	**% reduction**
304299	47
420915	76
420921	50
420922	54
420950	87
420957	50
420959	50

[0441] RBP4 protein levels were also measured in the plasma using an ELISA kit. Plasma samples were taken predose (on day -5) and on days 9, 16, 23, 30, 44, 58, 72, and 86. The results are presented in Table 74 expressed as percentage inhibition compared to the predose levels. Some of the ISIS oligonucleotides (ISIS 420915, ISIS 420922, ISIS 420950, ISIS 420955 and ISIS 420959) demonstrate a time-dependent reduction in protein levels, concomitant with TTR reduction. The final plasma RBP4 levels are presented in Table 75 and also demonstrate the strong correlation between RBP4 and TTR protein level reductions (Table 73) on treatment with the above-mentioned oligonucleotides. Specifically, treatment with ISIS 420915 caused greater inhibition of RBP4 plasma protein than treatment with ISIS 304299 (63% inhibition vs. 19% inhibition), even though the two oligonucleotides differ from each other by a single base-pair shift.

Table 74

Time course of RBP4 protein level reduction in the cynomolgus monkey plasma relative to predose levels								
ISIS No.	**Day 9**	**Day 16**	**Day 23**	**Day 30**	**Day 44**	**Day 58**	**Day 72**	**Day 86**
304299	0	6	10	4	1	9	13	19
420915	5	22	22	30	38	47	54	63
420921	0	0	0	0	0	0	6	24
420922	4	19	16	34	33	29	15	32
420950	30	44	46	47	52	54	47	48
420955	6	36	53	65	n/a	n/a	n/a	n/a
420957	0	10	0	0	0	0	3	27
420959	18	22	14	17	19	25	22	34
n/a= study was terminated on day 31 for animals treated with ISIS 420955; therefore data for subsequent days is not available.								

Table 75

Day 86 RBP4 protein level reduction in the cynomolgus monkey plasma relative to predose levels	
ISIS No.	**% reduction**
304299	19
420915	63
420921	24
420922	32
420950	48
420957	27
420959	34

Tolerability studies

Body and organ weight measurements

[0442] To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body and organ weights were measured at day 86. The data for animals treated with ISIS 420955 was taken at day 31. Body weights were measured and compared to that at pre-dose levels. Organ weights were measured and treatment group weights were compared to the corresponding PBS control weights. The data is presented in Table 76.

Table 76

Final body and organ weight % changes in the cynomolgus monkey relative to predose levels				
ISIS No.	**Body weight**	**Liver weight**	**Kidney weight**	**Spleen weight**
304299	+6	+27	+37	+53
420915	+6	+37	+26	+41
420921	+4	+42	+43	+22
420922	+4	+45	+39	+63
420950	0	+204	+166	+297
420955*	-3	+36	+81	+70
420957	-6	+55	+184	+109
420959	0	+57	+101	+112
(*Data of day 31)				

Liver function

[0443] To evaluate the effect of ISIS oligonucleotides on hepatic function, blood samples were collected from all the study groups. The blood samples were collected in tubes without any anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Concentrations of transaminases were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured on day 86 and the results are presented in Table 77, expressed in IU/L. Alkaline phosphatase, which is synthesized in increased amounts by damaged liver cells, is also a marker of liver disease and was similarly measured. C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, was also similarly measured on day 86. Both alkaline phosphatase and CRP data are also presented in Table 77. Bilirubin is also a liver metabolic marker and was similarly measured and is presented in Table 77, expressed in mg/dL.

Table 77

Effect of antisense oligonucleotide treatment on liver metabolic markers in cynomolgus monkey plasma					
	AST (IU/L)	**ALT (IU/L)**	**ALP (IU/L)**	**CRP (mg/L)**	**Bilirubin (m/dL)**
PBS	60	54	955	2.4	0.24
ISIS 304299	81	101	747	3.3	0.17
ISIS 420915	68	62	672	1.6	0.15
ISIS 420921	98	107	832	3.2	0.14
ISIS 420922	94	96	907	2.4	0.15
ISIS 420950	132	94	1032	12.9	0.11
ISIS 420957	100	73	868	23.5	0.15
ISIS 420959	70	63	811	16.0	0.13

Kidney function

[0444] To evaluate the effect of ISIS oligonucleotides on kidney function, blood samples were collected from all the study groups. The blood samples were collected in tubes without any anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Concentrations of BUN and creatinine were measured at day 86 using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in Table 78, expressed in mg/dL.

[0445] Urine samples were collected by drainage from special stainless-steel cage pans on day 5 before the study, and subsequently on days 25 and 84. Urine total protein to creatinine ratio was measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan) and the results are presented in Table 79.

Table 78

Effect of antisense oligonucleotide treatment on plasma BUN and creatinine levels (mg/dL) in cynomolgus monkeys		
	BUN	**Creatinine**
PBS	28	0.86
ISIS 304299	27	0.85
ISIS 420915	25	0.90
ISIS 420921	33	0.99
ISIS 420922	28	0.86
ISIS 420950	36	0.97
ISIS 420957	35	0.86
ISIS 420959	27	0.89

Table 79

Effect of antisense oligonucleotide treatment on urine protein to creatine ratio in cynomolgus monkeys			
	Day -5	**Day 25**	**Day 84**
PBS	0.003	0.01	0.00
ISIS 304299	0.000	0.01	0.00
ISIS 420915	0.003	0.00	0.00
ISIS 420921	0.033	0.13	0.09
ISIS 420922	0.010	0.05	0.02

(continued)

Effect of antisense oligonucleotide treatment on urine protein to creatine ratio in cynomolgus monkeys			
	Day -5	**Day 25**	**Day 84**
ISIS 420950	0.008	0.29	0.21
ISIS 420955	0.000	0.61	n/a
ISIS 420957	0.000	0.48	0.36
ISIS 420959	0.005	0.08	0.03
n/a= study was terminated on day 31 for animals treated with ISIS 420955; therefore data for subsequent days is not available.			

Hematology

[0446] To evaluate any inflammatory effect of ISIS oligonucleotides in cynomolgus monkeys, blood samples were approximately 0.5 mL of blood was collected from each of the available study animals in tubes containing the potassium salt of EDTA. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, individual white blood cell percentages, such as that of monocytes, neutrophils, lymphocytes, as well as for platelet count and hematocrit (%), using an ADVIA120 hematology analyzer (Bayer, USA). The data is presented in Table 80.

Table 80

Effect of antisense oligonucleotide treatment on hematologic parameters in cynomolgus monkeys							
	WBC (x $10^3/\mu L$)	**RBC (x $10^6/\mu L$)**	**Platelet (x 1000/μL)**	**Hematocrit (%)**	**Lymphocytes (%)**	**Neutrophil (%)**	**Monocytes (%)**
PBS	9.6	5.3	415	40	62	35	1.8
ISIS 304299	11.6	5.2	395	38	68	26	3.1
ISIS 420915	10.3	5.1	382	36	72	22	3.5
ISIS 420921	9.8	5.2	385	36	60	34	2.5
ISIS 420922	11.6	5.2	396	37	62	29	5.4
ISIS 420950	13.7	4.4	260	33	51	34	7.8
ISIS 420957	18.6	4.7	298	33	52	35	9.1
ISIS 420959	7.7	4.8	306	32	62	29	5.5

Analysis of factors of inflammation

[0447] To evaluate the effect of ISIS oligonucleotides on factors involved in inflammation, blood was collected on day 86 from all available animals for complement C3 analysis, as well as for measurement of cytokine levels. For complement C3 analysis, the blood samples were collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Complement C3 was measured using an automatic analyzer (Toshiba 200 FR NEO chemistry analyzer, Toshiba co., Japan). The data is presented in Table 81, expressed in mg/dL.

[0448] For cytokine level analyses, blood was collected in tubes containing EDTA for plasma separation. The tubes were then centrifuged (3000 rpm for 10 min at room temperature) to obtain plasma. Plasma samples were sent to Aushon Biosystems Inc. (Billerica, MA) for measurement of chemokine and cytokine levels. Levels of TNF-α were measured

using the respective primate antibodies and levels of MIP-1α, MCP-1, and MIP-1β were measured using the respective cross-reacting human antibodies. Measurements were taken 5 days before the start of treatment and on days 3 and 86. The results are presented in Tables 82-85.

Table 81

Effect of antisense oligonucleotide treatment on complement C3 (mg/dL) in cynomolgus monkeys	
	C3
PBS	133
ISIS 304299	96
ISIS 420915	104
ISIS 420921	91
ISIS 420922	102
ISIS 420950	70
ISIS 420957	69
ISIS 420959	95

Table 82

Effect of antisense oligonucleotide treatment on MCP-1 (pg/mL) in cynomolgus monkeys			
	Day -5	**Day 3**	**Day 86**
PBS	232	362	206
ISIS 304299	219	292	427
ISIS 420915	204	342	400
ISIS 420921	281	407	2120
ISIS 420922	215	482	838
ISIS 420950	170	370	3355
ISIS 420957	208	308	3485
ISIS 420959	237	715	2035

Table 83

Effect of antisense oligonucleotide treatment on TNF-α (pg/mL) in cynomolgus monkeys			
	Day -5	**Day 3**	**Day 86**
PBS	60	46	16
ISIS 304299	46	35	24
ISIS 420915	113	83	30
ISIS 420921	57	50	56
ISIS 420922	30	59	46
ISIS 420950	48	54	266
ISIS 420957	29	33	87
ISIS 420959	22	77	74

Table 84

Effect of antisense oligonucleotide treatment on MIP-1α (pg/mL) in cynomolgus monkeys			
	Day -5	**Day 3**	**Day 86**
PBS	6	7	7
ISIS 304299	6	7	9
ISIS 420915	5	5	10
ISIS 420921	8	11	9
ISIS 420922	9	8	5
ISIS 420950	7	9	5
ISIS 420957	6	6	6
ISIS 420959	9	6	5

Table 85

Effect of antisense oligonucleotide treatment on MIP-1β (pg/mL) in cynomolgus monkeys			
	Day -5	**Day 3**	**Day 86**
PBS	13	19	42
ISIS 304299	17	23	54
ISIS 420915	15	27	72
ISIS 420921	23	43	112
ISIS 420922	9	41	70
ISIS 420950	8	25	126
ISIS 420957	16	27	182
ISIS 420959	36	46	117

Coagulation tests

[0449] To evaluate the effect of ISIS oligonucleotides on factors involved in the coagulation pathway, the standard tests for coagulation were employed. PT and aPTT were measured using platelet poor plasma (PPP) from the monkeys over a period of 48 hrs. PT and aPTT values are provided in Tables 86 and 87 and expressed in seconds. Fibrinogen levels on the plasma were also quantitated over a period of 48 hrs and the data is presented in Table 88. As shown in Tables 86-88, PT, aPTT and fibrinogen were not significantly altered in monkeys treated with ISIS oligonucleotides compared to the PBS control.

Table 86

Effect of antisense oligonucleotide treatment on PT (sec)						
	0 hr	**1 hr**	**4 hr**	**8 hr**	**24 hr**	**48 hr**
PBS	10.08	10.38	10.10	10.33	9.83	9.40
ISIS 304299	10.38	10.30	10.48	10.20	9.95	9.53
ISIS 420915	10.15	10.13	10.38	9.93	9.75	9.48
ISIS 420921	10.28	10.13	10.43	10.18	9.80	9.55
ISIS 420922	9.95	10.00	10.05	9.70	9.48	9.28
ISIS 420950	10.30	10.47	10.57	10.27	9.63	9.50

(continued)

Effect of antisense oligonucleotide treatment on PT (sec)						
	0 hr	**1 hr**	**4 hr**	**8 hr**	**24 hr**	**48 hr**
ISIS 420957	10.63	10.47	10.60	10.77	10.33	10.27
ISIS 420959	10.08	10.10	10.20	10.15	9.80	9.55

Table 87

Effect of antisense oligonucleotide treatment on aPTT (sec)						
	0 hr	**1 hr**	**4 hr**	**8 hr**	**24 hr**	**48 hr**
PBS	19.40	19.70	20.13	20.20	19.43	17.30
ISIS 304299	21.83	24.35	27.05	25.73	22.40	18.78
ISIS 420915	20.05	22.83	23.83	24.00	21.78	17.90
ISIS 420921	24.15	26.68	31.78	31.90	27.80	22.15
ISIS 420922	25.28	29.48	34.83	33.90	29.13	25.08
ISIS 420950	28.13	31.40	35.40	35.40	31.40	28.37
ISIS 420957	29.13	33.27	39.13	37.40	36.50	29.93
ISIS 420959	22.45	24.73	29.18	28.38	25.50	20.65

Table 88

Effect of antisense oligonucleotide treatment on fibrinogen (mg/dL)						
	0 hr	**1 hr**	**4 hr**	**8 hr**	**24 hr**	**48 hr**
PBS	212	203	240	247	282	272
ISIS 304299	175	172	198	207	227	200
ISIS 420915	213	196	204	258	257	215
ISIS 420921	208	209	230	237	301	249
ISIS 420922	278	277	335	338	400	304
ISIS 420950	293	295	348	376	390	296
ISIS 420957	280	299	344	330	434	328
ISIS 420959	276	277	354	326	414	320

Thyroid panel analysis

[0450] To evaluate the effect of ISIS oligonucleotides on thyroid hormones, monkeys were fasted overnight and 3.5 mL of blood was drawn from each of the available study animals 5 days prior to the start of treatment and on days 51 and 86. Collected blood samples were kept in tubes without anticoagulant for serum separation. The tubes were kept for 90 min at room temperature, after which they were centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Serum samples were sent to the Biomarkers Core Laboratory of Emory University (Atlanta, GA) for thyroid panel analysis. The results for thyroid stimulating hormone (TSH) are provided in Table 89 and expressed μL/mL. The results for total and free T3 hormone are provided in Tables 90 and 91.The results for total and free T4 hormone are provided in Tables 92 and 93. Overall, the thyroid panel analysis showed that all the animals remained within acceptable hormone levels even though transthyretin expression levels were reduced, demonstrating that the transthyretin antisense oligonucleotides did not affect hormone levels.

Table 89

Effect of antisense oligonucleotide treatment on TSH (μL/mL			
	Day -5	**Day 51**	**Day 86**
PBS	0.8	0.7	1.0
ISIS 304299	1.4	1.0	2.2
ISIS 420915	1.4	1.5	2.5
ISIS 420921	0.7	0.6	1.0
ISIS 420922	1.0	1.2	1.9
ISIS 420950	0.6	2.2	5.4
ISIS 420957	0.6	2.6	4.9
ISIS 420959	0.9	1.6	4.7

Table 90

Effect of antisense oligonucleotide treatment on total T3 (ng/dL)			
	Day -5	**Day 51**	**Day 86**
PBS	177	248	140
ISIS 304299	202	226	176
ISIS 420915	156	206	156
ISIS 420921	217	204	137
ISIS 420922	188	177	131
ISIS 420950	260	208	105
ISIS 420957	266	160	78
ISIS 420959	299	219	137

Table 91

Effect of antisense oligonucleotide treatment on free T3 (pg/mL)			
	Day -5	**Day 51**	**Day 86**
PBS	7.7	5.8	5.2
ISIS 304299	9.2	6.0	4.7
ISIS 420915	8.9	5.6	4.5
ISIS 420921	10.2	4.8	4.0
ISIS 420922	8.9	5.4	3.7
ISIS 420950	7.2	3.8	2.1
ISIS 420957	8.8	4.0	2.4
ISIS 420959	8.3	4.9	3.3

Table 92

Effect of antisense oligonucleotide treatment on total T4 (ng/dL)			
	Day -5	**Day 51**	**Day 86**
PBS	5.8	4.9	4.4
ISIS 304299	8.1	5.5	6.1
ISIS 420915	8.3	5.7	5.5
ISIS 420921	7.6	6.1	5.6
ISIS 420922	7.3	6.1	5.8
ISIS 420950	6.1	6.3	5.7
ISIS 420957	6.3	4.4	5.0
ISIS 420959	7.9	5.9	8.1

Table 93

Effect of antisense oligonucleotide treatment on free T4 (pg/mL)			
	Day -5	**Day 51**	**Day 86**
PBS	3.4	2.4	1.7
ISIS 304299	3.2	2.5	1.7
ISIS 420915	5.0	1.8	1.7
ISIS 420921	2.6	1.5	1.5
ISIS 420922	3.5	1.6	1.5
ISIS 420950	2.5	1.2	1.1
ISIS 420957	2.4	1.2	1.2
ISIS 420959	3.8	1.4	1.5

Pharmacokinetic studies

Measurement of oligonucleotide concentration

[0451] The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenolchloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTT-GCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 166) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. The ratio of the concentrations in the kidney versus the liver was calculated. The results are presented in Tables 94 and 95, expressed as μg/g tissue.

Table 94

Full-length oligonucleotide concentration (μg/g) in the liver of cynomolgus monkey			
ISIS No.	**Kidney**	**Liver**	**Kidney/Liver ratio**
304299	2179	739	2.9
420915	2439	1064	2.3
420921	4617	1521	3.0
420922	3957	1126	3.5

(continued)

Full-length oligonucleotide concentration (μg/g) in the liver of cynomolgus monkey			
ISIS No.	**Kidney**	**Liver**	**Kidney/Liver ratio**
420950	3921	1082	3.6
420955	2444	1111	2.2
420957	3619	1230	2.9
420959	3918	1158	3.4

Table 95

Total oligonucleotide concentration (μg/g) in the liver of cynomolgus monkey			
ISIS No.	**Kidney**	**Liver**	**Kidney/Liver ratio**
304299	3098	992	3.1
420915	3024	1266	2.4
420921	6100	1974	3.1
420922	4861	1411	3.4
420950	6003	1553	3.9
420955	2763	1208	2.3
420957	5420	1582	3.4
420959	5498	1501	3.7

SEQUENCE LISTING

[0452]

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<120> MODULATION OF TRANSTHYRETIN EXPRESSION

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Claims

1. A compound comprising a modified oligonucleotide having a nucleobase sequence consisting of the 20 linked nucleosides recited in SEQ ID NO: 80.

2. The compound of claim 1, wherein the compound consists of a modified oligonucleotide having a nucleobase sequence consisting of the 20 linked nucleosides recited in SEQ ID NO: 80.

3. The compound of claim 1 or claim 2, wherein the modified oligonucleotide is a single-stranded oligonucleotide.

4. The compound of any one of claims 1-3, wherein at least one internucleoside linkage is a modified internucleoside linkage.

5. The compound of claim 4, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

6. The compound of any one of claims 1-5, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar.

7. The compound of claim 6, wherein the at least one modified sugar is a bicyclic sugar.

8. The compound of claim 7, wherein each of the at least one bicyclic sugar comprises a 4'-(CH_2)-O-2', 4'-$(CH_2)_2$-O-2', or a 4'-$CH(CH_3)$-O-2' bridge.

9. The compound of claim 6, wherein at least one modified sugar comprises a 2'-O-methoxyethyl group.

10. The compound of any one of claims 1-9, wherein at least one nucleoside comprises a modified nucleobase.

11. The compound of claim 10, wherein the modified nucleobase is a 5-methylcytosine.

12. The compound of any one of claims 1-11, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;
a 5' wing segment consisting of linked nucleosides; and
a 3' wing segment consisting of linked nucleosides;
wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

13. The compound of any one of claims 1-12, wherein the modified oligonucleotide comprises:

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

14. A composition comprising the compound of any one of claims 1-13 or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

15. A compound of any one of claims 1-13 or composition of claim 14 for use in treating transthyretin amyloidosis in a human.

16. The compound or composition for use of claim 15, wherein the use of the compound or composition reduces at least one of restlessness, lack of coordination, nystagmus, spastic paraparesis, lack of muscle coordination, impaired vision, insomnia, unusual sensations, myoclonus, blindness, loss of speech, Carpal tunnel syndrome, seizures, subarachnoid hemorrhages, stroke and bleeding in the brain, hydrocephalus, ataxia, and spastic paralysis, coma, sensory neuropathy, parathesia, hypesthesia, motor neuropathy, autonomic neuropathy, orthostatic hypotension, cyclic constipation, cyclic diarrhea, nausea, vomiting, reduced sweating, impotence, delayed gastric emptying, urinary retention, urinary incontinence, progressive cardiopathy, fatigue, shortness of breath, weight loss, lack of appetite, numbness, tingling, weakness, enlarged tongue, nephrotic syndrome, congestive heart failure, dyspnea on exertion, peripheral edema, arrhythmias, palpitations, light-headedness, syncope, postural hypotension, peripheral nerve problems, sensory motor impairment, lower limb neuropathy, upper limb neuropathy, hyperalgesia, altered temperature sensation, lower extremity weakness, cachexia, peripheral edema, hepatomegaly, purpura, diastolic dysfunction, premature ventricular contractions, cranial neuropathy, diminished deep tendon reflexes, amyloid deposits in the corpus vitreum, vitreous opacity, dry eyes, glaucoma, scalloped appearance in the pupils, or swelling of the feet due to water retention in the human.

17. The compound or composition for use of claim 15, wherein the transthyretin amyloidosis is familial amyloid polyneuropathy (FAP).

18. The compound or composition for use of claim 15, wherein the transthyretin amyloidosis is familial amyloid cardiomyopathy (FAC).

Patentansprüche

1. Verbindung, umfassend ein modifiziertes Oligonukleotid mit einer aus den 20 verknüpften Nukleosiden gemäß SEQ ID NO: 80 bestehenden Nukleobasensequenz.

2. Verbindung nach Anspruch 1, wobei die Verbindung aus einem modifizierten Oligonukleotid mit einer aus den 20 verknüpften Nukleosiden gemäß SEQ ID NO: 80 bestehenden Nukleobasensequenz besteht.

3. Verbindung nach Anspruch 1 oder Anspruch 2, wobei es sich bei dem modifizierten Oligonukleotid um ein einzelsträngiges Oligonukleotid handelt.

4. Verbindung nach einem der Ansprüche 1-3, wobei es sich bei wenigstens einer Internukleosidverknüpfung um eine modifizierte Internukleosidverknüpfung handelt.

5. Verbindung nach Anspruch 4, wobei es sich bei den Internukleosidverknüpfungen jeweils um eine Phosphorothioat-Internukleosidverknüpfung handelt.

6. Verbindung nach einem der Ansprüche 1-5, wobei wenigstens ein Nukleosid des modifizierten Oligonukleotids einen modifizierten Zucker umfasst.

7. Verbindung nach Anspruch 6, wobei es sich bei dem wenigstens einen modifizierten Zucker um einen bicyclischen Zucker handelt.

8. Verbindung nach Anspruch 7, wobei der wenigstens eine bicyclische Zucker jeweils eine 4'-(CH_2)-O-2'-, 4'-$(CH_2)_2$-O-2'- oder eine 4'-$CH(CH_3)$-O-2'-Brücke umfasst.

9. Verbindung nach Anspruch 6, wobei wenigstens ein modifizierter Zucker eine 2'-O-Methoxyethyl-Gruppe umfasst.

10. Verbindung nach einem der Ansprüche 1-9, wobei wenigstens ein Nukleosid eine modifizierte Nukleobase umfasst.

11. Verbindung nach Anspruch 10, wobei es sich bei der modifizierten Nukleobase um ein 5-Methylcytosin handelt.

12. Verbindung nach einem der Ansprüche 1-11, wobei das modifizierte Oligonukleotid Folgendes umfasst:

ein Gap-Segment, das aus verknüpften Desoxynukleosiden besteht;
ein 5'-Wing-Segment, das aus verknüpften Nukleosiden besteht; und
ein 3'-Wing-Segment, das aus verknüpften Nukleosiden besteht;
wobei das Gap-Segment zwischen dem 5'-Wing-Segment und dem 3'-Wing-Segment positioniert ist und wobei jedes Nukleosid eines jeden Wing-Segments einen modifizierten Zucker umfasst.

13. Verbindung nach einem der Ansprüche 1-12, wobei das modifizierte Oligonukleotid Folgendes umfasst:

ein Gap-Segment, das aus zehn verknüpften Desoxynukleosiden besteht;
ein 5'-Wing-Segment, das aus fünf verknüpften Nukleosiden besteht; und
ein 3'-Wing-Segment, das aus fünf verknüpften Nukleosiden besteht;
wobei das Gap-Segment zwischen dem 5'-Wing-Segment und dem 3'-Wing-Segment positioniert ist, wobei jedes Nukleosid eines jeden Wing-Segments einen 2'-O-Methoxyethyl-Zucker umfasst, wobei es sich bei den Cytosinen des modifizierten Oligonukleotids jeweils um ein 5-Methylcytosin handelt und wobei es sich bei den Internukleosidverknüpfungen des modifizierten Oligonukleotids jeweils um eine Phosphorothioat-Verknüpfung handelt.

14. Zusammensetzung, umfassend die Verbindung nach einem der Ansprüche 1-13 oder Salz davon und ein pharmazeutisch unbedenkliches Träger- oder/und Verdünnungsmittel.

15. Verbindung nach einem der Ansprüche 1-13 oder Zusammensetzung nach Anspruch 14 zur Verwendung bei der Behandlung von Transthyretin-Amyloidose bei einem Menschen.

16. Verbindung oder Zusammensetzung zur Verwendung nach Anspruch 15, wobei die Verwendung der Verbindung oder Zusammensetzung wenigstens eines der Folgenden reduziert: Unruhe, Koordinationsmangel, Nystagmus, spastische Paraplegie, mangelnde Muskelkoordination, beeinträchtigtes Sehvermögen, Schlaflosigkeit, ungewöhnliche Sinneseindrücke, Myoklonie, Blindheit, Sprachverlust, Karpaltunnelsyndrom, Krampfanfälle, Subarachnoidalblutungen, Schlaganfall und Gehirnblutung, Hydrocephalus, Ataxie und spastische Lähmung, Koma, sensorische Neuropathie, Parästhesie, Hypästhesie, motorische Neuropathie, autonome Neuropathie, orthostatische Hypotonie, periodische Verstopfung, periodischer Durchfall, Übelkeit, Erbrechen, verminderte Schweißbildung, Impotenz, verzögerte Magenentleerung, Harnretention, Harninkontinenz, progressive Kardiopathie, Müdigkeit, Kurzatmigkeit, Gewichtsverlust, Appetitlosigkeit, Taubheitsgefühl, Kribbeln, Asthenie, vergrößerte Zunge, nephrotisches Syndrom, kongestive Herzinsuffizienz, Dyspnoe bei Belastung, peripheres Ödem, Arrhythmien, Herzklopfen, Schwindelgefühl, Synkope, posturale Hypotonie, periphere Nervenprobleme, sensorisch-motorische Beeinträchtigung, Neuropathie der unteren Gliedmaßen, Neuropathie der oberen Gliedmaßen, Hyperalgesie, veränderte Temperaturwahrnehmung, Schwäche der unteren Extremitäten, Kachexie, peripheres Ödem, Hepatomegalie, Purpura, diastolische Fehlfunktion, vorzeitige ventrikuläre Kontraktionen, kraniale Neuropathie, verminderte tiefe Sehnenreflexe, Amyloidablagerungen im Glaskörper, Glaskörpertrübung, trockene Augen, Glaukom, muschelartiges Erscheinungsbild in

den Pupillen oder Anschwellen der Füße aufgrund von Wasseransammlung beim Menschen.

17. Verbindung oder Zusammensetzung zur Verwendung nach Anspruch 15, wobei es sich bei der Transthyretin-Amyloidose um familiäre Amyloid-Polyneuropathie (FAP) handelt.

18. Verbindung oder Zusammensetzung zur Verwendung nach Anspruch 15, wobei es sich bei der Transthyretin-Amyloidose um familiäre Amyloid-Kardiomyopathie (Familial Amyloid Cardiomyopathy, FAC) handelt.

Revendications

1. Composé comprenant un oligonucléotide modifié ayant une séquence de nucléobases constituée des 20 nucléosides liés décrits dans SEQ ID NO: 80.

2. Composé de la revendication 1, le composé étant constitué d'un oligonucléotide modifié ayant une séquence de nucléobases constituée des 20 nucléosides liés décrits dans SEQ ID NO: 80.

3. Composé de la revendication 1 ou la revendication 2, dans lequel l'oligonucléotide modifié est un oligonucléotide simple brin.

4. Composé de l'une quelconque des revendications 1 à 3, dans lequel au moins un lieur internucléosidique est un lieur internucléosidique modifié.

5. Composé de la revendication 4, dans lequel chaque un lieur internucléosidique est un lieur internucléosidique phosphorothioate.

6. Composé de l'une quelconque des revendications 1 à 5, dans lequel au moins un nucléoside de l'oligonucléotide modifié comprend un sucre modifié.

7. Composé de la revendication 6, dans lequel l'au moins un sucre modifié est un sucre bicyclique.

8. Composé de la revendication 7, dans lequel chacun des au moins un sucre bicyclique comprend un pont 4'-(CH_2)-O-2', 4'-$(CH_2)_2$-O-2', ou 4'-$CH(CH_3)$-O-2'.

9. Composé de la revendication 6, dans lequel au moins un sucre modifié comprend un groupe 2'-O-méthoxyéthyle.

10. Composé de l'une quelconque des revendications 1 à 9, dans lequel au moins un nucléoside comprend une nucléobase modifiée.

11. Composé de la revendication 10, dans lequel la nucléobase modifiée est une 5-méthylcytosine.

12. Composé de l'une quelconque des revendications 1 à 11, dans lequel l'oligonucléotide modifié comprend :

    un segment de brèche constitué de désoxynucléosides liés ;
    un segment d'aile en 5' constitué de nucléosides liés ;
    et
    un segment d'aile en 3' constitué de nucléosides liés ; dans lequel le segment de brèche est positionné entre le segment d'aile en 5' et le segment d'aile en 3' et dans lequel chaque nucléoside de chaque segment d'aile comprend un sucre modifié.

13. Composé de l'une quelconque des revendications 1 à 12, dans lequel l'oligonucléotide modifié comprend :

    un segment de brèche constitué de dix désoxynucléosides liés ;
    un segment d'aile en 5' constitué de cinq nucléosides liés ; et
    un segment d'aile en 3' constitué de cinq nucléosides liés ;
    dans lequel le segment de brèche est positionné entre le segment d'aile en 5' et le segment d'aile en 3', où chaque nucléoside de chaque segment d'aile comprend un 2'-O-méthoxyéthyl-sucre ; dans lequel chaque cytosine de l'oligonucléotide modifié est une 5-méthylcytosine, et dans lequel chaque lieur internucléosidique

de l'oligonucléotide modifié est un lieur phosphorothioate.

14. Composition comprenant le composé de l'une quelconque des revendications 1 à 13 ou un sel de celui-ci et au moins l'un d'un véhicule ou diluant pharmaceutiquement acceptable.

15. Composé de l'une quelconque des revendications 1 à 13 ou composition de la revendication 14 pour utilisation dans le traitement de l'amylose de la transthyrétine chez un humain.

16. Composé ou composition pour utilisation de la revendication 15, l'utilisation du composé ou de la composition réduisant au moins l'un parmi l'agitation, un déficit de coordination, le nystagmus, la paraparésie spastique, un déficit de coordination musculaire, un trouble de la vision, l'insomnie, des sensations inhabituelles, la myoclonie, la cécité, la perte de la parole, le syndrome du canal carpien, les crises épileptiques, les hémorragies sous-arachnoïdiennes, un accident vasculaire cérébral et un saignement dans le cerveau, l'hydrocéphalie, l'ataxie et la paralysie spastique, un coma, une neuropathie sensorielle, la parathésie, l'hypesthésie, une neuropathie motrice, une neuropathie autonome, l'hypotension orthostatique, la constipation cyclique, la diarrhée cyclique, la nausée, le vomissement, une transpiration réduite, l'impuissance, une vidange gastrique retardée, la rétention urinaire, l'incontinence urinaire, une cardiopathie progressive, la fatigue, l'essoufflement, la perte de poids, le manque d'appétit, un engourdissement, des picotements, une faiblesse, une hypertrophie de la langue, le syndrome néphrotique, l'insuffisance cardiaque congestive, la dyspnée de l'effort, un oedème périphérique, des arythmies, des palpitations, des étourdissements, une syncope, une hypotension posturale, des problèmes des nerfs périphériques, un trouble sensoriel moteur, une neuropathie des membres inférieurs, une neuropathie des membres supérieurs, l'hyperalgésie, une altération de sensation à la température, une faiblesse des extrémités inférieures, la cachexie, un oedème périphérique, l'hépatomégalie, le purpura, un dysfonctionnement diastolique, des contractions ventriculaires prématurées, une neuropathie cranienne, une diminution des réflexes des tendons profonds, des dépôts amyloïdes dans le corps vitré, une opacité vitréenne, la sécheresse oculaire, le glaucome, un aspect dentelé dans les pupilles, ou un gonflement des pieds dû à la rétention d'eau chez l'humain.

17. Composé ou composition pour utilisation de la revendication 15, l'amylose de la transthyrétine étant une polyneuropathie amyloïde familiale (FAP).

18. Composé ou composition pour utilisation de la revendication 15, l'amylose de la transthyrétine étant une cardiomyopathie amyloïde familiale (FAC).

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 20080039418 A **[0007]**
- US 20070299027 A **[0007]**
- WO 2010017509 A **[0008]**
- WO 2010048228 A **[0014]**
- WO 2008101157 A **[0222]**
- US 20050130923 A **[0222]**
- WO 2007134181 A **[0222] [0225]**
- US 7399845 B **[0224] [0225]**
- WO 2009006478 A **[0224] [0225]**
- WO 2008150729 A **[0224] [0225]**
- US 20040171570 A **[0224]**
- US 7427672 B **[0224]**
- WO 2008154401 A **[0224] [0225]**
- US 6268490 B **[0225]**
- US 6525191 B **[0225]**
- US 6670461 B **[0225]**
- US 6770748 B **[0225]**
- US 6794499 B **[0225]**
- US 7034133 B **[0225]**
- US 7053207 B **[0225]**
- US 7547684 B **[0225]**
- US 7696345 B **[0225]**
- US 20080039618 A **[0225]**
- US 20090012281 A **[0225]**
- US 60989574 B **[0225]**
- US 61026995 B **[0225]**
- US 61026998 B **[0225]**
- US 61056564 B **[0225]**
- US 61086231 B **[0225]**
- US 61097787 B **[0225]**
- US 61099844 B **[0225]**
- WO 1994014226 PCT **[0225]**
- WO 2004106356 A **[0225]**
- WO 2005021570 A **[0225]**
- DK 9800393 W **[0225]**
- WO 9914226 A **[0225] [0236] [0237]**
- WO 9839352 A **[0236]**
- WO 03004602 A **[0263]**
- US 20050244869 A **[0281]**
- US 20090082300 A **[0281]**
- US 5108921 A **[0318]**
- US 5354844 A **[0318]**
- US 5416016 A **[0318]**
- US 5459127 A **[0318]**
- US 5521291 A **[0318]**
- US 5543158 A **[0318]**
- US 5547932 A **[0318]**
- US 5583020 A **[0318]**
- US 5591721 A **[0318]**
- US 4426330 A **[0318]**
- US 4534899 A **[0318]**
- US 5013556 A **[0318]**
- US 5213804 A **[0318]**
- US 5227170 A **[0318]**
- US 5264221 A **[0318]**
- US 5356633 A **[0318]**
- US 5395619 A **[0318]**
- US 5417978 A **[0318]**
- US 5462854 A **[0318]**
- US 5469854 A **[0318]**
- US 5512295 A **[0318]**
- US 5527528 A **[0318]**
- US 5534259 A **[0318]**
- US 5543152 A **[0318]**
- US 5556948 A **[0318]**
- US 5580575 A **[0318]**
- US 5595756 A **[0318]**
- US 6287860 B **[0320] [0326] [0328] [0331] [0332]**
- WO 61329538 A **[0452]**
- WO 61405163 A **[0452]**

Non-patent literature cited in the description

- **SAKAKI et al.** *Mol Biol Med.,* 1989, vol. 6, 161-8 **[0002]**
- **PALHA.** *Clin Chem Lab Med,* 2002, vol. 40, 1292-1300 **[0003]**
- **DICKSON et al.** *J Biol Chem,* 1986, vol. 261, 3475-3478 **[0003]**
- **TANSKANEN et al.** *Ann Med.,* 2008, vol. 40 (3), 232-9 **[0004]**
- **COSTA et al.** *Proc. Natl. Acad. Sci. USA,* 1978, vol. 75, 4499-4503 **[0005]**
- **SARAIVA et al.** *J. Clin. Invest.,* 1984, vol. 74, 104-119 **[0005]**
- **SOUSA ; SARAIVA.** *Prog. Neurobiol.,* 2003, vol. 71, 385-400 **[0005]**
- **BENSON et al.** *Muscle & Nerve,* 2006, vol. 33 (5), 609-618 **[0009]**
- **SEKIJIMA et al.** *Current Pharmaceutical Design,* 2008, vol. 14 (30), 3219-3230 **[0010]**

- **KUROSAWA et al.** *Biochemical and Biophysical Research Communications,* 2005, vol. 337 (3), 1012-1018 **[0011]**
- **TASAKI et al.** *Amyloid: The International Journal of Experimental and Clinical Investigation,* 2010, vol. 17 (1), 52-53 **[0012]**
- **BENSON et al.** *Muscle & Nerve,* 2007, vol. 36 (4), 411-423 **[0013]**
- **WOOLF et al.** *Proc. Natl. Acad. Sci. USA,* 1992, vol. 89, 7305-7309 **[0180]**
- **GAUTSCHI et al.** *J. Natl. Cancer Inst.,* March 2001, vol. 93, 463-471 **[0181]**
- **MAHER ; DOLNICK.** *Nuc. Acid. Res.,* 1988, vol. 16, 3341-3358 **[0182]**
- **ALTSCHUL et al.** *J. Mol. Biol.,* 1990, vol. 215, 403-410 **[0208]**
- **ZHANG ; MADDEN.** *Genome Res.,* 1997, vol. 7, 649-656 **[0208]**
- Wisconsin Sequence Analysis Package. Genetics Computer Group. University Research Park **[0208]**
- **SMITH ; WATERMAN.** *Adv. Appl. Math.,* 1981, vol. 2, 482-489 **[0208]**
- **CHATTOPADHYAYA et al.** *J. Org. Chem.,* 2009, vol. 74, 118-134 **[0224]**
- **SINGH et al.** *Chem. Commun.,* 1998, vol. 4, 455-456 **[0225]**
- **KOSHKIN et al.** *Tetrahedron,* 1998, vol. 54, 3607-3630 **[0225] [0236]**
- **WAHLESTEDT et al.** *Proc. Natl. Acad. Sci. U. S. A.,* 2000, vol. 97, 5633-5638 **[0225]**
- **KUMAR et al.** *Bioorg. Med. Chem. Lett.,* 1998, vol. 8, 2219-2222 **[0225] [0237]**
- **SINGH et al.** *J. Org. Chem.,* 1998, vol. 63, 10035-10039 **[0225] [0237]**
- **SRIVASTAVA et al.** *J. Am. Chem. Soc.,* 2007, vol. 129 (26), 8362-8379 **[0225] [0239]**
- **ELAYADI et al.** *Curr. Opinion Invest. Drugs,* 2001, vol. 2, 558-561 **[0225]**
- **BRAASCH et al.** *Chem. Biol.,* 2001, vol. 8, 1-7 **[0225]**
- **ORUM et al.** *Curr. Opinion Mol. Ther.,* 2001, vol. 3, 239-243 **[0225]**
- **FRIEDEN et al.** *Nucleic Acids Research,* 2003, vol. 21, 6365-6372 **[0228]**
- **FREIER et al.** *Nucleic Acids Research,* 1997, vol. 25 (22), 4429-4443 **[0239]**
- **ALBAEK et al.** *J. Org. Chem.,* 2006, vol. 71, 7731-7740 **[0239]**
- **BAKER et al.** *J. Biol. Chem.,* 1997, vol. 272, 11944-12000 **[0242]**
- **MARTIN.** *Helv. Chim. Acta,* 1995, vol. 78, 486-504 **[0242]**
- **ALTMANN et al.** *Chimia,* 1996, vol. 50, 168-176 **[0242]**
- **ALTMANN et al.** *Biochem. Soc. Trans.,* 1996, vol. 24, 630-637 **[0242]**
- **ALTMANN et al.** *Nucleosides Nucleotides,* 1997, vol. 16, 917-926 **[0242]**
- **LEUMANN.** *Bioorg. Med. Chem.,* 2002, vol. 10, 841-854 **[0243] [0250]**
- Antisense Research and Applications. CRC Press, 1993, 276-278 **[0254]**
- **JONES, L.J. et al.** *Analytical Biochemistry,* 1998, vol. 265, 368-374 **[0277]**

TRANSZTIRETIN EXPRESSZIÓJÁNAK MÓDOSÍTÁSA

Szabadalmi igénypontok

1. Vegyület, amely a SEQ ID NO: 80 szerinti 20 kapcsolt nukleozidból álló nukleobázis-szekvenciájú módosított oligonukleotidot tartalmaz.

2. Az 1. igénypont szerinti vegyület, ahol a vegyület a SEQ ID NO: 80 szerinti 20 kapcsolt nukleozidból álló nukleobázis-szekvenciájú módosított oligonukleotidból áll.

3. Az 1. vagy 2. igénypont szerinti vegyület, ahol a módosított oligonukleotid egyszálú oligonukleotid.

4. Az 1-3. igénypontok bármelyike szerinti vegyület, ahol legalább egy internukleozid kötés módosított internukleozid kötés.

5. 4. igénypont szerinti vegyület, ahol mindegyik internukleozid kötés foszforotioát internukleozid kötés.

6. Az 1-5. igénypontok bármelyike szerinti vegyület, ahol a módosított oligonukleotid legalább egy nukleozidja módosított cukrot tartalmaz.

7. A 6. igénypont szerinti vegyület, ahol a legalább egy módosított cukor biciklusos cukor.

8. A 7. igénypont szerinti vegyület, ahol a legalább egy biciklusos cukor mindegyike 4'-(CH_2)-O-2', 4'-(CH_2)$_2$-O-2' vagy 4'-CH(CH_3)-O-2' hidat tartalmaz.

9. A 6. igénypont szerinti vegyület, ahol legalább egy módosított cukor tartalmaz 2'-O-metoxietil csoportot.

10. Az 1-9. igénypontok bármelyike szerinti vegyület, ahol legalább egy nukleozid tartalmaz módosított nukleobázist.

11. A 10. igénypont szerinti vegyület, ahol a módosított nukleobázis 5-metilcitozin.

12. Az 1-11. igénypontok bármelyike szerinti vegyület, ahol a módosított oligonukleotid:
kapcsolt dezoxinukleozidokból álló „rés" („gap") szegmenst,
kapcsolt nukleozidokból álló 5' „szárny" („wing") szegmenst és
kapcsolt nukleozidokból álló 3' „szárny" („wing") szegmenst
tartalmaz, ahol a „rés" szegmens az 5' „szárny" szegmens és a 3' „szárny" szegmens között helyezkedik el, és ahol mindegyik „szárny" szegmens mindegyik nukleozidja tartalmaz módosított cukrot.

13. Az 1-12. igénypontok bármelyike szerinti vegyület, ahol a módosított oligonukleotid:
tíz kapcsolt dezoxinukleozidból álló „rés" szegmenst,
öt kapcsolt nukleozidból álló 5' „szárny" szegmenst és
öt kapcsolt nukleozidból álló 3' „szárny" szegmenst
tartalmaz, ahol a „rés" szegmens az 5' „szárny" szegmens és a 3' „szárny" szegmens között helyezkedik el, ahol mindegyik „szárny" szegmensben lévő mindegyik nukleozid tartalmaz 2'-O-metoxietil cukrot, ahol a módosított oligonukleotidban lévő mindegyik citozin 5-metilcitozin, és ahol a módosított oligonukleotidban mindegyik internukleozid kötés foszforotioát kötés.

14. Készítmény, amely 1-13. igénypontok bármelyike szerinti vegyületet vagy annak sóját és legalább egy gyógyászatilag elfogadható hordozót vagy hígítót tartalmaz.

15. Az 1-13. igénypontok bármelyike szerinti vegyület vagy a 14. igénypont szerinti készítmény emberben transztiretin-amiloidózis kezelésében történő alkalmazásra.

16. A 15. igénypont szerinti vegyület vagy készítmény az ott meghatározott alkalmazásra, ahol a vegyület vagy készítmény alkalmazása a következők legalább egyikét csökkenti az emberben: nyugtalanság, koordináció hiánya, szemtekerezgés, spasztikus paraparézis, izomkoordináció hiánya, látáskárosodás, álmatlanság, szokatlan érzet, izomrángás, vakság, beszédképesség elvesztése, kéztőalagút szindróma, görcsök, szubarachnoid vérzés, agyi érkatasztrófa és vérzés az agyban, agykamratágulat, ataxia és spasztikus bénulás, kóma, szenzoros neuropátia, paresztézia, hipesztézia, motoros neuropátia, autonóm neuropátia, ortosztatikus hipotenzió, ciklusos székrekedés, ciklusos hasmenés, émelygés, hányás, csökkent izzadás, impotencia, késleltetett gyomorürülés, vizeletretenció, vizelet inkontinencia, progresszív kardiopátia, fáradtság, légszomj, súlycsökkenés, étvágytalanság, kábaság, bizsergés, gyengeség, megnagyobbodott nyelv, nefrotikus szindróma, pangásos szívelégtelenség, terheléses dyspnoe, perifériás ödéma, ritmuszavarok, palpitáció, szédülés, eszméletvesztés, poszturális hipotenzió, perifériás idegproblémák, szenzoros-motoros károsodás, alsóvégtag-neuropátia, felsővégtag-neuropátia, hiperalgézia, megváltozott hőmérsékletérzékelés, alsó végtag gyengesége, cachexia, perifériás ödéma, hepatomegália, purpura, diasztolés rendellenesség, korai kamrai összehúzódások, kraniális neuropátia, csökkent mély ínreflexek, amiloidlerakódás a corpus vitreumban, üvegtest-homály, száraz szem, glaukóma, csipkés jelenség a pupillában, vagy vízvisszatartás miatt a láb dagadása.

17. A 15. igénypont szerinti vegyület vagy készítmény az ott meghatározott alkalmazásra, ahol transztiretin amiloidózis familiáris amiloid polineuropátia (FAP).

18. A 15. igénypont szerinti vegyület vagy készítmény az ott meghatározott alkalmazásra, ahol transztiretin amiloidózis familiáris amiloid kardiomiopátia (FAC).