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(54) Titre : MODULATEURS SPECIFIQUES ALLELIQUES DE LA RHODOPSINE P23H
(54) Title: ALLELE SPECIFIC MODULATORS OF P23H RHODOPSIN

(57) Abrégé/Abstract:
The present embodiments provide methods, compounds, and compositions for treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) by administering a P23H rhodopsin specific inhibitor to a subject. The present embodiments provided herein are directed to compounds and compositions useful for treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP). In certain embodiments, P23H rhodopsin inhibitors provided herein are allele-specific antisense compounds targeted to a P23H mutant allele that are capable of selectively inhibiting expression of P23H rhodopsin mutant protein to a greater extent than wild-type protein. In certain embodiments, administration of the allele specific antisense compounds in a subject having AdRP results in selective inhibition of P23H rhodopsin and allows the normal protein produced from the wild-type allele to maintain rod survival and function in the subject.

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(54) **Title**: ALLELE SPECIFIC MODULATORS OF P23H RHODOPSIN

(57) **Abstract**: The present embodiments provide methods, compounds, and compositions for treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) by administering a P23H rhodopsin specific inhibitor to a subject. The present embodiments provided herein are directed to compounds and compositions useful for treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP). In certain embodiments, P23H rhodopsin inhibitors provided herein are allele-specific antisense compounds targeted to a P23H mutant allele that are capable of selectively inhibiting expression of P23H rhodopsin mutant protein to a greater extent than wild-type protein. In certain embodiments, administration of the allele specific antisense compounds in a subject having AdRP results in selective inhibition of P23H rhodopsin and allows the normal protein produced from the wild-type allele to maintain rod survival and function in the subject.

ALLELE SPECIFIC MODULATORS OF P23H RHODOPSIN

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0267WOSEQ_ST25.txt created February 26, 2016, which is 60 kb in size.

Field

The present embodiments provide methods, compounds, and compositions for treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) by administering a P23H rhodopsin specific inhibitor to a subject.

Background

Retinitis pigmentosa (RP) is a broad description for pigment changes and/or damage in the retina. A hereditary form of retinitis pigmentosa called autosomal dominant retinitis pigmentosa (AdRP) is a degenerative disease that typically causes blindness by middle age. Bird AC, *American journal of ophthalmology* 1995;119:543-562; Boughman JA et al. *Am J Hum Genet* 1980;32:223-235; Schuster A et al. *Br J Ophthalmol* 2005;89:1258-1264. AdRP is caused by abnormalities of the photoreceptors (rods and cones) or the retinal pigment epithelium (RPE) of the retina leading to progressive sight loss. AdRP patients may experience defective light to dark, dark to light adaptation or night blindness as the result of the degeneration of the peripheral visual field. AdRP results in loss of photoreceptor (rods) cells from peripheral retina and then cones from central retina.

Over 100 rhodopsin mutations have been identified in patients with AdRP. Sullivan LS et al. *Invest Ophthalmol Vis Sci* 2006;47:3052-3064; Wang DY et al. *Clinica chimica acta; international journal of clinical chemistry* 2005;351:5-16. The P23H mutation is the most prevalent mutation and is present in ~25% of AdRP and 5-15% of RP cases. Dryja TP et al. *Proc Natl Acad Sci USA* 1991;88:9370-9374. Mutant rhodopsin protein such as P23H has a toxic gain-of-function that induces misfolding and disruption of normal rhodopsin protein, which leads to photoreceptor cell apoptosis. Typically, rods degenerate first, affecting low light vision. Then, cones degenerate, affecting bright light and color vision. The age of onset is variable with gradual progressive reduction in night and peripheral vision, often leading to "gun-barrel" visual field or tunnel vision. Median age of night-blindness onset is 12-14 years old. Blindness is frequent in middle ages and most rod cells are lost by age 40.

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Summary

The present embodiments provided herein are directed to potent, tolerable, and/or selective compounds and compositions useful for treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP). In certain embodiments, P23H rhodopsin inhibitors provided herein are allele-specific antisense compounds targeted to a P23H mutant allele that are capable of selectively inhibiting expression of P23H rhodopsin mutant protein to a greater extent than wild-type protein. In certain embodiments, administration of the allele-specific antisense compounds in a subject having AdRP results in selective inhibition of P23H rhodopsin and allows the normal protein produced from the wild-type allele to maintain rod survival and function in the subject.

Detailed Description

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

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

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 #) indicate a combination of nucleobase sequence, chemical modification, and motif.

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 at the 2' position of a sugar ring, e.g. a furanose ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

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

“2’-substituted nucleoside” means a nucleoside comprising a substituent at the 2’-position of the furanosyl ring other than H or OH. In certain embodiments, 2’ substituted nucleosides include nucleosides with bicyclic sugar modifications.

“3’ target site” refers to the nucleotide of a target nucleic acid which is complementary to the 3’-most nucleotide of a particular antisense compound.

“5’ target site” refers to the nucleotide of a target nucleic acid which is complementary to the 5’-most nucleotide of a particular antisense compound.

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

“About” means within ±10% of a value. For example, if it is stated, “the compounds affected at least about 70% inhibition of P23H rhodopsin, it is implied that P23H rhodopsin levels are inhibited within a range of 60% and 80%.

"Administration" or "administering" refers to routes of introducing an antisense compound provided herein to a subject to perform its intended function. An example of a route of administration that can be used includes, but is not limited to intravitreal administration.

“Allele specific” with respect to an inhibitor refers to an inhibitor, such as an antisense compound, designed to hybridize to and/or inhibit expression of a transcript from one allele of a gene to a greater extent than the other allele of the gene.

“Amelioration” refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. In certain embodiments, amelioration includes a delay or slowing in the progression of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

“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.

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

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

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

“Antisense mechanisms” are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

“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.

“Base complementarity” refers to the capacity for the precise base pairing of nucleobases of an antisense oligonucleotide with corresponding nucleobases in a target nucleic acid (i.e., hybridization), and is mediated by Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen binding between corresponding nucleobases.

“Bicyclic sugar moiety” means a modified sugar moiety comprising a 4 to 7 membered ring (including but not limited to a furanosyl) comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In certain embodiments, the 4 to 7 membered ring is a sugar ring. In certain embodiments the 4 to 7 membered ring is a furanosyl. In certain such embodiments, the bridge connects the 2’-carbon and the 4’-carbon of the furanosyl.

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

“cEt” or “constrained ethyl” means a bicyclic sugar moiety comprising a bridge connecting the 4’-carbon and the 2’-carbon, wherein the bridge has the formula: 4’-CH(CH_3)-O-2’.

“Constrained ethyl nucleoside” (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4’-CH(CH_3)-O-2’ bridge.

“P23H rhodopsin” means any nucleic acid or protein of P23H rhodopsin. “P23H rhodopsin nucleic acid” means any nucleic acid encoding P23H rhodopsin. For example, in certain embodiments, a P23H rhodopsin nucleic acid includes a DNA sequence encoding P23H rhodopsin, an RNA sequence transcribed from DNA encoding P23H rhodopsin (including genomic DNA comprising introns and exons), and an mRNA sequence encoding P23H rhodopsin. “P23H rhodopsin mRNA” means an mRNA encoding a P23H rhodopsin protein.

“P23H rhodopsin specific inhibitor” refers to any agent capable of specifically inhibiting P23H rhodopsin RNA and/or P23H rhodopsin protein expression or activity at the molecular level. For example, P23H rhodopsin specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of P23H rhodopsin RNA and/or P23H rhodopsin protein.

“Chemically distinct region” refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2’-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2’-O-methoxyethyl modifications.

“Chimeric antisense compounds” means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

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

"Comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

“Contiguous nucleobases” means nucleobases immediately adjacent to each other.

“Deoxyribonucleotide” means a nucleotide having a hydrogen at the 2’ position of the sugar portion of the nucleotide. Deoxyribonucleotides may be modified with any of a variety of substituents.

“Designing” or “Designed to” refer to the process of designing an oligomeric compound that specifically hybridizes with a selected nucleic acid molecule.

“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.

“Efficacy” means the ability to produce a desired effect.

“Expression” includes all the functions by which a gene’s coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to the products of transcription and translation.

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

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

“Hybridization” means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a nucleic acid target. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense oligonucleotide and a nucleic acid target.

“Identifying an animal having, or at risk for having, a disease, disorder and/or condition” means identifying an animal having been diagnosed with the disease, disorder and/or condition or identifying an animal predisposed to develop the disease, disorder and/or condition. Such identification may be accomplished by any method including evaluating an individual’s medical history and standard clinical tests or assessments.

“Immediately adjacent” means there are no intervening elements between the immediately adjacent elements.

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

"Inhibiting the expression or activity" refers to a reduction, blockade of the expression or activity and does not necessarily indicate a total elimination of expression or activity.

“Internucleoside linkage” refers to the chemical bond between nucleosides.

“Lengthened” antisense oligonucleotides are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein.

“Linked deoxynucleoside” means a nucleic acid base (A, G, C, T, U) substituted by deoxyribose linked by a phosphate ester to form a nucleotide.

“Linked nucleosides” means adjacent nucleosides linked together by an internucleoside linkage.

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

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

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

"Modified nucleoside" means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase.

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

"Modified oligonucleotide" means an oligonucleotide comprising at least one modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

"Modified sugar" means substitution and/or any change from a natural sugar moiety.

"Modulating" refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating P23H rhodopsin mRNA can mean to increase or decrease the level of P23H rhodopsin mRNA and/or P23H rhodopsin protein in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, a P23H rhodopsin antisense compound can be a modulator that decreases the amount of P23H rhodopsin mRNA and/or P23H rhodopsin protein in a cell, tissue, organ or organism.

"Monomer" refers to a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides, whether naturally occuring or modified.

"Motif" means the pattern of unmodified and modified nucleosides in an antisense compound.

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

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

"Non-complementary nucleobase" refers to a pair of nucleobases that do not form hydrogen bonds with one another or otherwise support hybridization.

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes, but is not limited to, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, and double-stranded nucleic acids.

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

"Nucleobase complementarity" refers to a nucleobase that is capable of base pairing with another nucleobase. For example, in DNA, adenine (A) is complementary to thymine (T). For example, in RNA, adenine (A) is complementary to uracil (U). In certain embodiments, complementary nucleobase refers to a nucleobase of an antisense compound that is capable of base pairing with a nucleobase of its target nucleic acid. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair.

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

"Nucleoside" means a nucleobase linked to a sugar.

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

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

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

"Oligonucleoside" means an oligonucleotide in which the internucleoside linkages do not contain a phosphorus atom.

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

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

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

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

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

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

"Prophylactically effective amount" refers to an amount of a pharmaceutical agent that provides a prophylactic or preventative benefit to an animal.

"Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

"Ribonucleotide" means a nucleotide having a hydroxy at the 2' position of the sugar portion of the nucleotide. Ribonucleotides may be modified with any of a variety of substituents.

"Segments" are defined as smaller or sub-portions of regions within a target nucleic acid.

"Selective" with respect to an effect refers to a greater effect on one thing over another by any quantitative extent or fold-difference. For example, an antisense compound that is "selective" for P23H rhodopsin or "selectively" targets or inhibits P23H rhodopsin, reduces expression of the P23H rhodopsin allele to a greater extent than the wild-type allele.

"Side effects" means physiological disease and/or conditions 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.

“Sites,” as used herein, are defined as unique nucleobase positions within a target nucleic acid.

"Slows progression" means decrease in the development of the said disease.

“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. “Stringent hybridization conditions” or “stringent conditions” refer to conditions under which an oligomeric compound will hybridize to its target sequence, but to a minimal number of other sequences.

“Specifically inhibit” a target nucleic acid means to reduce or block expression of the target nucleic acid while exhibiting fewer, minimal, or no effects on non-target nucleic acids and does not necessarily indicate a total elimination of the target nucleic acid’s expression.

“Subject” means a human or non-human animal selected for treatment or therapy.

“Target” refers to a protein, the modulation of which is desired.

“Target gene” refers to a gene encoding a target.

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

“Target nucleic acid,” “target RNA,” “target RNA transcript” and “nucleic acid target” all mean a nucleic acid capable of being targeted by antisense compounds.

“Target region” means a portion of a target nucleic acid to which one or more antisense compounds is targeted.

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

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

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

"Unmodified" nucleobases mean the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

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

Certain Embodiments

Certain embodiments provide methods, compounds and compositions for inhibiting or selectively inhibiting P23H rhodopsin expression.

Certain embodiments provide antisense compounds targeted to a P23H rhodopsin nucleic acid. In certain embodiments, the human mutant P23H rhodopsin nucleic acid has a C to A substitution at nucleotide 163 of GENBANK Accession No. NM_000539.3 and is incorporated herein as SEQ ID NO: 2. In certain embodiments, the human mutant P23H rhodopsin nucleic acid has a C to A substitution in codon 23 (exon 1) of a human rhodopsin gene having the sequence of GENBANK Accession No. NM_000539.3. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

Certain embodiments provide an antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

Certain embodiments provide an antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 9 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

Certain embodiments provide an antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

Certain embodiments provide an antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 11 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

Certain embodiments provide an antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

Certain embodiments provide an antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

Certain embodiments provide an antisense compound comprising a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the antisense compound is a single-stranded oligonucleotide.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 8 to 80 linked nucleosides having at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion complementary to an equal length portion within nucleotides 157-174, 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2.

In certain embodiments, a compound comprises a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within nucleotides 157-174, 157-174, 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 8 to 80 linked nucleosides having a nucleobase sequence comprising at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 9 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 10 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 11 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64.

In certain embodiments, a modified oligonucleotide targeted to P23H rhodopsin is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. Out of over 400 antisense oligonucleotides that were screened as described in the Examples section below, ISIS 564426, ISIS 664844, ISIS 664867, and ISIS 664884 emerged as the top lead compounds. In particular, ISIS 664844 exhibited the best combination of properties in terms of potency, tolerability, and selectivity for P23H rhodopsin out of over 400 antisense oligonucleotides.

In certain embodiments, any of the foregoing compounds or oligonucleotides comprises at least one modified internucleoside linkage, at least one modified sugar, and/or at least one modified nucleobase.

In certain embodiments, any of the foregoing compounds or oligonucleotides comprises at least one modified sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group. In certain embodiments, at least one modified sugar is a bicyclic sugar, such as a 4'-$CH(CH_3)$-O-2' group, a 4'-CH_2-O-2' group, or a 4'-$(CH_2)_2$-O-2'group.

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

In certain embodiments, any of the foregoing compounds or oligonucleotides comprises at least one modified nucleobase, such as 5-methylcytosine.

In certain embodiments, any of the foregoing compounds or oligonucleotides 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. In certain embodiments, the oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 15, 44, or 52.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 11-64, 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.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64, 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.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 15, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three 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 cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 15, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5’ wing segment consisting of three linked nucleosides; and

a 3’ wing segment consisting of three 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 cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 64, wherein the modified oligonucleotide comprises:

a gap segment consisting of nine linked deoxynucleosides;

a 5’ wing segment consisting of four linked nucleosides; and

a 3’ wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5’ wing segment and the 3’ wing segment; wherein the 5’ wing segment comprises a cEt sugar, a cEt sugar, a cEt sugar, and a 2'-flouro sugar in the 5’ to 3’ direction; wherein each nucleoside of the 3’ wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 64, wherein the modified oligonucleotide comprises:

a gap segment consisting of nine linked deoxynucleosides;

a 5’ wing segment consisting of four linked nucleosides; and

a 3’ wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5’ wing segment and the 3’ wing segment; wherein the 5’ wing segment comprises a cEt sugar, a cEt sugar, a cEt sugar, and a 2'-flouro sugar in the 5’ to 3’ direction; wherein each nucleoside of the 3’ wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 21, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5’ wing segment consisting of two linked nucleosides; and a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, a cEt sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 21, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, a cEt sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 15 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 29, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, and a cEt sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 15 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 29, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, and a cEt sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing embodiments, the compound or oligonucleotide can be at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% complementary to a nucleic acid encoding P23H rhodopsin.

In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide. In certain embodiments, the compound comprises deoxyribonucleotides.

In any of the foregoing embodiments, the antisense compound can be double-stranded. In certain embodiments, a compound comprises ribonucleotides.

In certain embodiments, compounds are capable of selectively targeting or inhibiting expression of the Rhodopsin P23H mutant allele. In certain embodiments, compounds have at least about a 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, or 20-fold selectivity for inhibiting expression of the Rhodopsin P23H mutant allele over the wild-type allele.

In certain embodiments, compounds or compositions provided herein comprise a salt of the modified oligonucleotide. In certain embodiments, the salt is a sodium salt. In certain embodiments, the salt is a potassium salt.

Certain embodiments provide a composition comprising the compound of any of the aforementioned embodiments or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition has a viscosity less than about 40 centipoise (cP), less than about 30 centipose (cP), less than about 20 centipose (cP), less than about 15 centipose (cP), or less than about 10 centipose (cP). In certain embodiments, the composition having any of the aforementioned viscosities comprises a compound provided herein at a concentration of about 100 mg/mL, about 125 mg/mL, about 150 mg/mL, about 175 mg/mL, about 200 mg/mL, about 225 mg/mL, about 250 mg/mL, about 275 mg/mL, or about 300 mg/mL. In certain embodiments, the composition having any of the aforementioned viscosities and/or compound concentrations has a temperature of room temperature or about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, or about 30°C.

Certain Indications

Certain embodiments provided herein relate to methods of treating, preventing, ameliorating, or slowing progression of a disease associated with P23H rhodopsin in a subject by administration of a P23H rhodopsin specific inhibitor, such as an antisense compound targeted to P23H rhodopsin. In certain embodiments, the inhibitor is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. Examples of diseases associated with P23H rhodopsin treatable, preventable, and/or ameliorable with the methods provided herein include retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP).

In certain embodiments, a method of treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP) or autosomal dominant retinitis pigmentosa (AdRP) in a subject comprises administering to the subject a P23H rhodopsin specific inhibitor, thereby treating, preventing, ameliorating, or slowing progression of retinitis pigmentosa (RP) or autosomal dominant retinitis pigmentosa (AdRP) in the subject. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound targeted to P23H rhodopsin, such as an antisense oligonucleotide targeted to P23H rhodopsin. In certain embodiments, the antisense compound is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide. In certain embodiments, the antisense compound is administered to the subject by intravitreally such as by intravitreal injection. In certain embodiments, administering the antisense compound improves, preserves, or prevents worsening of visual function; visual field; photoreceptor cell function; electroretinogram (ERG) response such as full field ERG measuring retina wide function, dark adapted ERG measuring scotopic rod function, or light adapted ERG measuring photopic cone function; visual acuity; and/or vision-related quality of life. In certain embodiments, administering the antisense compound inhibits, prevents, or delays progression of photoreceptor cell loss and/or deterioration of the retina outer nuclear layer (ONL). In certain embodiments, the subject is identified as having the P23H rhodopsin mutant allele.

In certain embodiments, a method of inhibiting expression of P23H rhodopsin in a subject having a P23H rhodopsin mutant allele comprises administering a P23H rhodopsin specific inhibitor to the subject, thereby inhibiting expression of P23H rhodopsin in the subject. In certain embodiments, administering the inhibitor inhibits expression of P23H rhodopsin in the eye, retina, peripheral retina, rod photoreceptors, and/or cones. In certain embodiments, the subject has, or is at risk of having retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP). In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound allele-specific for P23H Rhodospin that selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide. In certain embodiments, the antisense compound is administered to the subject by intravitreally such as by intravitreal injection.

In certain embodiments, a method of improving or preserving visual function, visual field, photoreceptor cell function, ERG response, or visual acuity in a subject having a P23H rhodopsin mutant allele or having retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP), comprises administering a P23H rhodopsin specific inhibitor to the subject. In certain embodiments, a method of inhibiting, preventing, or delaying progression of photoreceptor cell loss and/or deterioration of the retina outer nuclear layer (ONL) in a subject having a P23H rhodopsin mutant allele or having retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP), comprises administering a P23H rhodopsin specific inhibitor to the subject. In certain embodiments, the inhibitor is an antisense compound targeted to P23H rhodopsin. In certain embodiments, the antisense compound is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS

664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide. In certain embodiments, the antisense compound is administered to the subject by intravitreally such as by intravitreal injection.

In certain embodiments, a method of inhibiting expression of P23H rhodopsin in a cell comprises contacting the cell with a P23H rhodopsin specific inhibitor to the subject. In certain embodiments, the cell is a rod photoreceptor cell or cone cell. In certain embodiments, the cell is in the eye of a subject. In certain embodiments, the cell is in the retina of the eye. In certain embodiments, the inhibitor is an antisense compound targeted to P23H rhodopsin. In certain embodiments, the antisense compound is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide.

Certain embodiments are drawn to a P23H rhodopsin specific inhibitor for use in treating retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) associated with P23H rhodopsin. In certain embodiments, the inhibitor is an antisense compound targeted to P23H rhodopsin. In certain embodiments, the antisense compound is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide.

Certain embodiments are drawn to a P23H rhodopsin specific inhibitor for use in improving or preserving visual function, visual field, photoreceptor cell function, ERG response, visual acuity, and/or vision-related quality of life of a subject having retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) associated with P23H rhodopsin. Certain embodiments are drawn to a P23H rhodopsin specific inhibitor for use in inhibiting, preventing, or delaying progression of photoreceptor cell loss and/or deterioration of the retina outer nuclear layer (ONL) in a subject having retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) associated with P23H rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide.

Certain embodiments are drawn to use of a P23H rhodopsin specific inhibitor for the manufacture of a medicament for treating retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) associated with P23H rhodopsin. In certain embodiments, the inhibitor is an antisense compound targeted to P23H rhodopsin. In certain embodiments, the antisense compound is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide.

Certain embodiments are drawn to use of a P23H rhodopsin specific inhibitor for the manufacture of a medicament for improving or preserving visual function, visual field, photoreceptor cell function, ERG response, visual acuity, and/or vision-related quality of life of a subject having retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) associated with P23H rhodopsin. Certain embodiments are drawn to use of a P23H rhodopsin specific inhibitor for the manufacture of a medicament for inhibiting, preventing, or delaying progression of photoreceptor cell loss and/or deterioration of the retina outer nuclear layer (ONL) in a subject having retinitis pigmentosa (RP), such as autosomal dominant retinitis pigmentosa (AdRP) associated with P23H rhodopsin. In certain embodiments, the inhibitor is an antisense compound targeted to P23H rhodopsin. In certain embodiments, the antisense compound is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 157-174, 157-171, 157-172, or 159-174 of SEQ ID NO: 2. In certain embodiments, the P23H rhodopsin specific inhibitor is a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 11-64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the P23H rhodopsin specific inhibitor is an antisense compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64. In certain embodiments, the

P23H rhodopsin specific inhibitor is ISIS 564426, ISIS 664844, ISIS 664867, or ISIS 664884. In any of the foregoing embodiments, the antisense compound can be a single-stranded oligonucleotide.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be an antisense compound targeted to P23H rhodopsin. In certain embodiments, the antisense compound is an antisense oligonucleotide, for example an antisense oligonucleotide consisting of 8 to 80 linked nucleosides, 12 to 30 linked nucleosides, or 20 linked nucleosides. In certain embodiments, the antisense oligonucleotide is at least 80%, 85%, 90%, 95% or 100% complementary to any of the nucleobase sequences recited in SEQ ID NOs: 1-4. In certain embodiments, the antisense oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar and/or at least one modified nucleobase. In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage, the modified sugar is a bicyclic sugar or a 2'-O-methoxyethyl, and the modified nucleobase is a 5-methylcytosine. In certain embodiments, 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 immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, the antisense oligonucleotide is allele-specific for P23H Rhodospin and selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 11-64, 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.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, 29, or 64, 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.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 15, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three 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 cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 15, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three 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 cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 64, wherein the modified oligonucleotide comprises:

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt sugar, a cEt sugar, a cEt sugar, and a 2'-flouro sugar in the 5' to 3' direction; wherein each nucleoside of the 3' wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 64, wherein the modified oligonucleotide comprises:

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt sugar, a cEt sugar, a cEt sugar, and a 2'-flouro sugar in the 5' to 3' direction; wherein each nucleoside of the 3' wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 21, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, a cEt sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 21, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, a cEt sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 15 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 29, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, and a cEt sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 15 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 29, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, and a cEt sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing methods or uses, the P23H rhodopsin specific inhibitor is administered intravitreally, such as by intravitreal injection.

Antisense compounds

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

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

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

In certain embodiments antisense oligonucleotides may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to an P23H rhodopsin nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

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

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

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

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

Certain Antisense Compound Motifs and Mechanisms

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

Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may confer another desired property e.g., serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense activity may result from any mechanism involving the hybridization of the antisense compound (e.g., oligonucleotide) with a target nucleic acid, wherein the hybridization ultimately results in a biological effect. In certain embodiments, the amount and/or activity of the target nucleic acid is modulated. In certain embodiments, the amount and/or activity of the target nucleic acid is reduced. In certain embodiments, hybridization of the antisense compound to the target nucleic acid ultimately results in target nucleic acid degradation. In certain embodiments, hybridization of the antisense compound to the target nucleic acid does not result in target nucleic acid degradation. In certain such embodiments, the presence of the antisense compound hybridized with the target nucleic acid (occupancy) results in a modulation of antisense activity. In certain embodiments, antisense compounds having a particular chemical motif or pattern of chemical modifications are particularly suited to exploit one or more mechanisms. In certain embodiments, antisense compounds function through more than one mechanism and/or through mechanisms that have not been elucidated. Accordingly, the antisense compounds described herein are not limited by particular mechanism.

Antisense mechanisms include, without limitation, RNase H mediated antisense; RNAi mechanisms, which utilize the RISC pathway and include, without limitation, siRNA, ssRNA and microRNA mechanisms; and occupancy based mechanisms. Certain antisense compounds may act through more than one such mechanism and/or through additional mechanisms.

RNase H-Mediated Antisense

In certain embodiments, antisense activity results at least in part from degradation of target RNA by RNase H. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNase H activity in mammalian cells. Accordingly, antisense compounds comprising at least a portion of DNA or DNA-like nucleosides may activate RNase H, resulting in cleavage of the target nucleic acid. In certain embodiments, antisense compounds that utilize RNase H comprise one or more modified nucleosides. In certain embodiments, such antisense compounds comprise at least one block of 1-8 modified nucleosides. In certain such embodiments, the modified nucleosides do not support RNase H activity. In certain embodiments, such antisense compounds are gapmers, as described herein. In certain such embodiments, the gap of the gapmer comprises DNA nucleosides. In certain such embodiments, the gap of the gapmer comprises DNA-like nucleosides. In certain such embodiments, the gap of the gapmer comprises DNA nucleosides and DNA-like nucleosides.

Certain antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a constrained ethyl). In certain embodiments, nucleosides in the wings may include several modified sugar moieties, including, for example 2'-MOE and bicyclic sugar moieties such as constrained ethyl or LNA. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides, bicyclic sugar moieties such as constrained ethyl nucleosides or LNA nucleosides, and 2'-deoxynucleosides.

Each distinct region may comprise uniform sugar moieties, variant, or alternating sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5'-wing, "Y" represents the length of the gap, and "Z" represents the length of the 3'-wing. "X" and "Z" may comprise uniform, variant, or alternating sugar moieties. In certain embodiments, "X" and "Y" may include one or more 2'-deoxynucleosides."Y" may comprise 2'-deoxynucleosides. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap is positioned immediately adjacent to each of the 5'-wing and the 3' wing. Thus, no intervening nucleotides exist between the 5'-wing and gap, or the gap and the 3'-wing. Any of the antisense compounds described herein can have a gapmer motif. In certain embodiments, "X" and "Z" are the same; in other embodiments they are different. In certain embodiments, "Y" is between 8 and 15 nucleosides. 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 nucleosides.

In certain embodiments, the antisense compound targeted to a P23H rhodopsin nucleic acid has a gapmer motif in which the gap consists of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 linked nucleosides.

In certain embodiments, the antisense oligonucleotide has a sugar motif described by Formula A as follows: $(J)_m$-$(B)_n$-$(J)_p$-$(B)_r$-$(A)_t$-$(D)_g$-$(A)_v$-$(B)_w$-$(J)_x$-$(B)_y$-$(J)_z$

wherein:

each A is independently a 2'-substituted nucleoside;

each B is independently a bicyclic nucleoside;

each J is independently either a 2'-substituted nucleoside or a 2'-deoxynucleoside;

each D is a 2'-deoxynucleoside;

m is 0-4; n is 0-2; p is 0-2; r is 0-2; t is 0-2; v is 0-2; w is 0-4; x is 0-2; y is 0-2; z is 0-4; g is 6-14;

provided that:

at least one of m, n, and r is other than 0;

at least one of w and y is other than 0;

the sum of m, n, p, r, and t is from 2 to 5; and

the sum of v, w, x, y, and z is from 2 to 5.

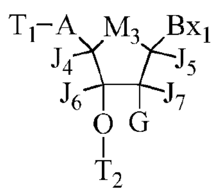
RNAi Compounds

In certain embodiments, antisense compounds are interfering RNA compounds (RNAi), which include double-stranded RNA compounds (also referred to as short-interfering RNA or siRNA) and single-stranded RNAi compounds (or ssRNA). Such compounds work at least in part through the RISC pathway to degrade and/or sequester a target nucleic acid (thus, include microRNA/microRNA-mimic compounds). In certain embodiments, antisense compounds comprise modifications that make them particularly suited for such mechanisms.

i. ssRNA compounds

In certain embodiments, antisense compounds including those particularly suited for use as single-stranded RNAi compounds (ssRNA) comprise a modified 5'-terminal end. In certain such embodiments, the 5'-terminal end comprises a modified phosphate moiety. In certain embodiments, such modified phosphate is stabilized (e.g., resistant to degradation/cleavage compared to unmodified 5'-phosphate). In certain embodiments, such 5'-terminal nucleosides stabilize the 5'-phosphorous moiety. Certain modified 5'-terminal nucleosides may be found in the art, for example in WO/2011/139702.

In certain embodiments, the 5'-nucleoside of an ssRNA compound has Formula IIc:



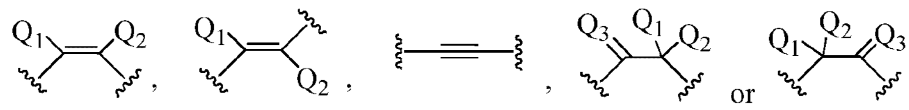
IIc

wherein:

T_1 is an optionally protected phosphorus moiety;

T_2 is an internucleoside linking group linking the compound of Formula IIc to the oligomeric compound;

A has one of the formulas:



Q_1 and Q_2 are each, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl or $N(R_3)(R_4)$;

Q_3 is O, S, $N(R_5)$ or $C(R_6)(R_7)$;

each R_3, R_4 R_5, R_6 and R_7 is, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl or C_1-C_6 alkoxy;

M_3 is O, S, NR_{14}, $C(R_{15})(R_{16})$, $C(R_{15})(R_{16})C(R_{17})(R_{18})$, $C(R_{15})$=$C(R_{17})$, $OC(R_{15})(R_{16})$ or $OC(R_{15})(Bx_2)$;

R_{14} is H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

R_{15}, R_{16}, R_{17} and R_{18} are each, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

Bx_1 is a heterocyclic base moiety;

or if Bx_2 is present then Bx_2 is a heterocyclic base moiety and Bx_1 is H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

J_4, J_5, J_6 and J_7 are each, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

or J_4 forms a bridge with one of J_5 or J_7 wherein said bridge comprises from 1 to 3 linked biradical groups selected from O, S, NR_{19}, $C(R_{20})(R_{21})$, $C(R_{20})$=$C(R_{21})$, $C[=C(R_{20})(R_{21})]$ and C(=O) and the other two of J_5, J_6 and J_7 are each, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

each R_{19}, R_{20} and R_{21} is, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

G is H, OH, halogen or $O-[C(R_8)(R_9)]_n-[(C=O)_m-X_1]_j-Z$;

each R_8 and R_9 is, independently, H, halogen, C_1-C_6 alkyl or substituted C_1-C_6 alkyl;

X_1 is O, S or $N(E_1)$;

Z is 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, substituted C_2-C_6 alkynyl or $N(E_2)(E_3)$;

E_1, E_2 and E_3 are each, independently, H, C_1-C_6 alkyl or substituted C_1-C_6 alkyl;

n is from 1 to about 6;

m is 0 or 1;

j is 0 or 1;

each substituted group comprises one or more optionally protected substituent groups independently selected from halogen, OJ_1, $N(J_1)(J_2)$, $=NJ_1$, SJ_1, N_3, CN, $OC(=X_2)J_1$, $OC(=X_2)N(J_1)(J_2)$ and $C(=X_2)N(J_1)(J_2)$;

X_2 is O, S or NJ_3;

each J_1, J_2 and J_3 is, independently, H or C_1-C_6 alkyl;

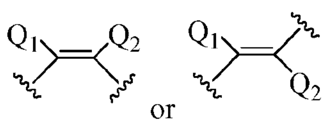
when j is 1 then Z is other than halogen or $N(E_2)(E_3)$; and

wherein said oligomeric compound comprises from 8 to 40 monomeric subunits and is hybridizable to at least a portion of a target nucleic acid.

In certain embodiments, M_3 is O, CH=CH, OCH_2 or $OC(H)(Bx_2)$. In certain embodiments, M_3 is O.

In certain embodiments, J_4, J_5, J_6 and J_7 are each H. In certain embodiments, J_4 forms a bridge with one of J_5 or J_7.

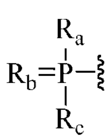
In certain embodiments, A has one of the formulas:



wherein:

Q_1 and Q_2 are each, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy or substituted C_1-C_6 alkoxy. In certain embodiments, Q_1 and Q_2 are each H. In certain embodiments, Q_1 and Q_2 are each, independently, H or halogen. In certain embodiments, Q_1 and Q_2 is H and the other of Q_1 and Q_2 is F, CH_3 or OCH_3.

In certain embodiments, T_1 has the formula:



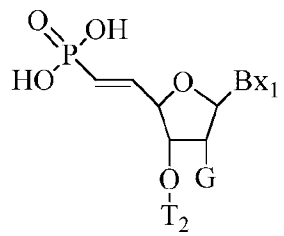
wherein:

R_a and R_c are each, independently, protected hydroxyl, protected thiol, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, protected amino or substituted amino; and

R_b is O or S. In certain embodiments, R_b is O and R_a and R_c are each, independently, OCH_3, OCH_2CH_3 or $CH(CH_3)_2$.

In certain embodiments, G is halogen, OCH_3, OCH_2F, $OCHF_2$, OCF_3, OCH_2CH_3, $O(CH_2)_2F$, OCH_2CHF_2, OCH_2CF_3, OCH_2-$CH{=}CH_2$, $O(CH_2)_2$-OCH_3, $O(CH_2)_2$-SCH_3, $O(CH_2)_2$-OCF_3, $O(CH_2)_3$-$N(R_{10})(R_{11})$, $O(CH_2)_2$-$ON(R_{10})(R_{11})$, $O(CH_2)_2$-$O(CH_2)_2$-$N(R_{10})(R_{11})$, $OCH_2C(=O)$-$N(R_{10})(R_{11})$, $OCH_2C(=O)$-$N(R_{12})$-$(CH_2)_2$-$N(R_{10})(R_{11})$ or $O(CH_2)_2$-$N(R_{12})$-$C(=NR_{13})[N(R_{10})(R_{11})]$ wherein R_{10}, R_{11}, R_{12} and R_{13} are each, independently, H or C_1-C_6 alkyl. In certain embodiments, G is halogen, OCH_3, OCF_3, OCH_2CH_3, OCH_2CF_3, OCH_2-$CH{=}CH_2$, $O(CH_2)_2$-OCH_3, $O(CH_2)_2$-$O(CH_2)_2$-$N(CH_3)_2$, $OCH_2C(=O)$-$N(H)CH_3$, $OCH_2C(=O)$-$N(H)$-$(CH_2)_2$-$N(CH_3)_2$ or OCH_2-$N(H)$-$C(=NH)NH_2$. In certain embodiments, G is F, OCH_3 or $O(CH_2)_2$-OCH_3. In certain embodiments, G is $O(CH_2)_2$-OCH_3.

In certain embodiments, the 5'-terminal nucleoside has Formula IIe:



IIe

In certain embodiments, antisense compounds, including those particularly suitable for ssRNA comprise one or more type of modified sugar moieties and/or naturally occurring sugar moieties arranged along an oligonucleotide or region thereof in a defined pattern or sugar modification motif. Such motifs may include any of the sugar modifications discussed herein and/or other known sugar modifications.

In certain embodiments, the oligonucleotides comprise or consist of a region having uniform sugar modifications. In certain such embodiments, each nucleoside of the region comprises the same RNA-like sugar modification. In certain embodiments, each nucleoside of the region is a 2'-F nucleoside. In certain embodiments, each nucleoside of the region is a 2'-OMe nucleoside. In certain embodiments, each nucleoside of the region is a 2'-MOE nucleoside. In certain embodiments, each nucleoside of the region is a cEt nucleoside. In certain embodiments, each nucleoside of the region is an LNA nucleoside. In certain embodiments, the uniform region constitutes all or essentially all of the oligonucleotide. In certain embodiments, the region constitutes the entire oligonucleotide except for 1-4 terminal nucleosides.

In certain embodiments, oligonucleotides comprise one or more regions of alternating sugar modifications, wherein the nucleosides alternate between nucleotides having a sugar modification of a first type and nucleotides having a sugar modification of a second type. In certain embodiments, nucleosides of both types are RNA-like nucleosides. In certain embodiments the alternating nucleosides are selected from: 2'-OMe, 2'-F, 2'-MOE, LNA, and cEt. In certain embodiments, the alternating modificatios are 2'-F and

2'-OMe. Such regions may be contiguous or may be interupted by differently modified nucleosides or conjugated nucleosides.

In certain embodiments, the alternating region of alternating modifications each consist of a single nucleoside (i.e., the patern is $(AB)_xA_y$ wheren A is a nucleoside having a sugar modification of a first type and B is a nucleoside having a sugar modification of a second type; x is 1-20 and y is 0 or 1). In certan embodiments, one or more alternating regions in an alternating motif includes more than a single nucleoside of a type. For example, oligonucleotides may include one or more regions of any of the following nucleoside motifs:

AABBAA;
ABBABB;
AABAAB;
ABBABAABB;
ABABAA;
AABABAB;
ABABAA;
ABBAABBABABAA;
BABBAABBABABAA; or
ABABBAABBABABAA;

wherein A is a nucleoside of a first type and B is a nucleoside of a second type. In certain embodiments, A and B are each selected from 2'-F, 2'-OMe, BNA, and MOE.

In certain embodiments, oligonucleotides having such an alternating motif also comprise a modified 5' terminal nucleoside, such as those of formula IIc or IIe.

In certain embodiments, oligonucleotides comprise a region having a 2-2-3 motif. Such regions comprises the following motif:

$$-(A)_2-(B)_x-(A)_2-(C)_y-(A)_3-$$

wherein: A is a first type of modifed nucleosde;

B and C, are nucleosides that are differently modified than A, however, B and C may have the same or different modifications as one another;

x and y are from 1 to 15.

In certain embodiments, A is a 2'-OMe modified nucleoside. In certain embodiments, B and C are both 2'-F modified nucleosides. In certain embodiments, A is a 2'-OMe modified nucleoside and B and C are both 2'-F modified nucleosides.

In certain embodiments, oligonucleosides have the following sugar motif:

$5'-(Q)-(AB)_xA_y-(D)_z$

wherein:

Q is a nucleoside comprising a stabilized phosphate moiety. In certain embodiments, Q is a nucleoside having Formula IIc or IIe;

A is a first type of modifed nucleoside;

B is a second type of modified nucleoside;

D is a modified nucleoside comprising a modification different from the nucleoside adjacent to it. Thus, if y is 0, then D must be differently modified than B and if y is 1, then D must be differently modified than A. In certain embodiments, D differs from both A and B.

X is 5-15;

Y is 0 or 1;

Z is 0-4.

In certain embodiments, oligonucleosides have the following sugar motif:

5'- (Q)- $(A)_x$-$(D)_z$

wherein:

Q is a nucleoside comprising a stabilized phosphate moiety. In certain embodiments, Q is a nucleoside having Formula IIc or IIe;

A is a first type of modifed nucleoside;

D is a modified nucleoside comprising a modification different from A.

X is 11-30;

Z is 0-4.

In certain embodiments A, B, C, and D in the above motifs are selected from: 2'-OMe, 2'-F, 2'-MOE, LNA, and cEt. In certain embodiments, D represents terminal nucleosides. In certain embodiments, such terminal nucleosides are not designed to hybridize to the target nucleic acid (though one or more might hybridize by chance). In certiain embodiments, the nucleobase of each D nucleoside is adenine, regardless of the identity of the nucleobase at the corresponding position of the target nucleic acid. In certain embodiments the nucleobase of each D nucleoside is thymine.

In certain embodiments, antisense compounds, including those particularly suited for use as ssRNA comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif. In certain embodiments, oligonucleotides comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least one 12 consecutive phosphorothioate internucleoside linkages. In certain such embodiments, at least one such block is located at the 3' end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide.

Oligonucleotides having any of the various sugar motifs described herein, may have any linkage motif. For example, the oligonucleotides, including but not limited to those described above, may have a linkage motif selected from non-limiting the table below:

5' most linkage	Central region	3'-region
PS	Alternating PO/PS	6 PS
PS	Alternating PO/PS	7 PS
PS	Alternating PO/PS	8 PS

ii. siRNA compounds

In certain embodiments, antisense compounds are double-stranded RNAi compounds (siRNA). In such embodiments, one or both strands may comprise any modification motif described above for ssRNA. In certain embodiments, ssRNA compounds may be unmodified RNA. In certain embodiments, siRNA compounds may comprise unmodified RNA nucleosides, but modified internucleoside linkages.

Several embodiments relate to double-stranded compositions wherein each strand comprises a motif defined by the location of one or more modified or unmodified nucleosides. In certain embodiments, compositions are provided comprising a first and a second oligomeric compound that are fully or at least partially hybridized to form a duplex region and further comprising a region that is complementary to and hybridizes to a nucleic acid target. It is suitable that such a composition comprise a first oligomeric compound that is an antisense strand having full or partial complementarity to a nucleic acid target and a second oligomeric compound that is a sense strand having one or more regions of complementarity to and forming at least one duplex region with the first oligomeric compound.

The compositions of several embodiments modulate gene expression by hybridizing to a nucleic acid target resulting in loss of its normal function. In certain embodiments, the degradation of the targeted P23H rhodopsin is facilitated by an activated RISC complex that is formed with compositions of the invention.

Several embodiments are directed to double-stranded compositions wherein one of the strands is useful in, for example, influencing the preferential loading of the opposite strand into the RISC (or cleavage) complex. The compositions are useful for targeting selected nucleic acid molecules and modulating the expression of one or more genes. In some embodiments, the compositions of the present invention hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA.

Certain embodiments are drawn to double-stranded compositions wherein both the strands comprises a hemimer motif, a fully modified motif, a positionally modified motif or an alternating motif. Each strand of the compositions of the present invention can be modified to fulfill a particular role in for example the siRNA pathway. Using a different motif in each strand or the same motif with different chemical modifications in each strand permits targeting the antisense strand for the RISC complex while inhibiting the incorporation of the sense strand. Within this model, each strand can be independently modified such that it is enhanced for its particular role. The antisense strand can be modified at the 5'-end to enhance its role in one region of the RISC while the 3'-end can be modified differentially to enhance its role in a different region of the RISC.

The double-stranded oligonucleotide molecules can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide molecules can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double-stranded structure, for example wherein the double-stranded region is about 15 to about 30, e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (e.g., about 15 to about 25 or more nucleotides of the double-stranded oligonucleotide molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the double-stranded oligonucleotide is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siRNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s).

The double-stranded oligonucleotide can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either in vivo or in vitro to generate an active siRNA molecule capable of mediating RNAi.

In certain embodiments, the double-stranded oligonucleotide comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the double-stranded oligonucleotide comprises nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the double-stranded oligonucleotide interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene.

As used herein, double-stranded oligonucleotides need not be limited to those molecules containing only RNA, but further encompasses chemically modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules lack 2'-hydroxy (2'-OH) containing nucleotides. In certain embodiments short interfering nucleic acids optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such double-stranded oligonucleotides that do not require the presence of ribonucleotides within the molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, double-stranded oligonucleotides can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, double-stranded oligonucleotides can be used to epigenetically silence genes at both the post-transcriptional level and the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siRNA molecules of the invention can result from siRNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel et al., 2004, Science, 303, 672-676; Pal-Bhadra et al., 2004, Science, 303, 669-672; Allshire, 2002, Science, 297, 1818-1819; Volpe et al., 2002, Science, 297, 1833-1837; Jenuwein, 2002, Science, 297, 2215-2218; and Hall et al., 2002, Science, 297, 2232-2237).

It is contemplated that compounds and compositions of several embodiments provided herein can target P23H rhodopsin by a dsRNA-mediated gene silencing or RNAi mechanism, including, e.g., "hairpin" or stem-loop double-stranded RNA effector molecules in which a single RNA strand with self-complementary sequences is capable of assuming a double-stranded conformation, or duplex dsRNA effector molecules comprising two separate strands of RNA. In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides and deoxynucleotides, such as the RNA/DNA hybrids disclosed, for example, by WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999. The dsRNA or dsRNA effector molecule may be a single molecule with a region of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In various embodiments, a dsRNA that consists of a single molecule consists entirely of ribonucleotides or includes a region of ribonucleotides that is complementary to a region of deoxyribonucleotides. Alternatively, the dsRNA may include two different strands that have a region of complementarity to each other.

In various embodiments, both strands consist entirely of ribonucleotides, one strand consists entirely of ribonucleotides and one strand consists entirely of deoxyribonucleotides, or one or both strands contain a mixture of ribonucleotides and deoxyribonucleotides. In certain embodiments, the regions of complementarity are at least 70, 80, 90, 95, 98, or 100% complementary to each other and to a target nucleic acid sequence. In certain embodiments, the region of the dsRNA that is present in a double-stranded conformation includes at least 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 75,100, 200, 500, 1000, 2000 or 5000 nucleotides or includes all of the nucleotides in a cDNA or other target nucleic acid sequence being represented in the dsRNA. In some embodiments, the dsRNA does not contain any single stranded regions, such as single stranded ends, or the dsRNA is a hairpin. In other embodiments, the dsRNA has one or more single stranded regions or overhangs. In certain embodiments, RNA/DNA hybrids include a DNA strand or region that is an antisense strand or region (e.g, has at least 70, 80, 90, 95, 98, or 100% complementarity to a target nucleic acid) and an RNA strand or region that is a sense strand or region (e.g, has at least 70, 80, 90, 95, 98, or 100% identity to a target nucleic acid), and vice versa.

In various embodiments, the RNA/DNA hybrid is made in vitro using enzymatic or chemical synthetic methods such as those described herein or those described in WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999. In other embodiments, a DNA strand synthesized in vitro is complexed with an RNA strand made in vivo or in vitro before, after, or concurrent with the transformation of the DNA strand into the cell. In yet other embodiments, the dsRNA is a single circular nucleic acid containing a sense and an antisense region, or the dsRNA includes a circular nucleic acid and either a second circular nucleic acid or a linear nucleic acid (see, for example, WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999.) Exemplary circular nucleic acids include lariat structures in which the free 5' phosphoryl group of a nucleotide becomes linked to the 2' hydroxyl group of another nucleotide in a loop back fashion.

In other embodiments, the dsRNA includes one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group) or contains an alkoxy group (such as a methoxy group) which increases the half-life of the dsRNA in vitro or in vivo compared to the corresponding dsRNA in which the corresponding 2' position contains a hydrogen or an hydroxyl group. In yet other embodiments, the dsRNA includes one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The dsRNAs may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the dsRNA contains one or two capped strands, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999.

In other embodiments, the dsRNA can be any of the at least partially dsRNA molecules disclosed in WO 00/63364, as well as any of the dsRNA molecules described in U.S. Provisional Application 60/399,998; and U.S. Provisional Application 60/419,532, and PCT/US2003/033466. Any of the dsRNAs may be expressed in vitro or in vivo using the methods described herein or standard methods, such as those described in WO 00/63364.

Occupancy

In certain embodiments, antisense compounds are not expected to result in cleavage or the target nucleic acid via RNase H or to result in cleavage or sequestration through the RISC pathway. In certain such embodiments, antisense activity may result from occupancy, wherein the presence of the hybridized antisense compound disrupts the activity of the target nucleic acid. In certain such embodiments, the antisense compound may be uniformly modified or may comprise a mix of modifications and/or modified and unmodified nucleosides.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode wild-type rhodopsin, without limitation, genomic sequence having the sequence set forth in GENBANK Accession No. NT_005612.16 truncated from

Date Reçue/Date Received 2021-07-06

nucleotides 35737800 to 35755500 (incorporated herein as SEQ ID NO: 1) and coding sequence having the sequence set forth in GENBANK Accession No NM_000539.3 (incorporated herein as SEQ ID NO: 3). Nucleotide sequences that encode mutant P23H rhodopsin nucleic acid have a C to A mutation at nucleotide 163 of GENBANK Accession No NM_000539.3 and is incorporated herein as SEQ ID NO: 2.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a P23H rhodopsin nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

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

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a P23H rhodopsin nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a P23H rhodopsin nucleic acid).

Non-complementary nucleobases between an antisense compound and a P23H rhodopsin nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a P23H rhodopsin nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a P23H rhodopsin nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

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

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a P23H rhodopsin nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

In certain embodiments, antisense compounds that are, or are up to 11, 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 P23H rhodopsin nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 11, 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 P23H rhodopsin nucleic acid, or specified portion thereof.

The antisense compounds provided 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 9 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least an 11 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 13 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

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

In certain embodiments, the antisense compounds, or portions thereof, are, or are at least, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

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

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

Modifications

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

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

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

Modified Internucleoside Linkages

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

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

In certain embodiments, antisense compounds targeted to a P23H rhodopsin nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substitutent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or $C(R_1)(R_2)$ (R, R_1 and R_2 are each independently H, C_1-C_{12} alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein 4'-(CH_2)-O-2' (LNA) is substituted with for example a 5'-methyl or a 5'-vinyl group).

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

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-CH(CH_3)-O-2' (also referred to as constrained ethyl or cEt) and 4'-CH(CH_2OCH_3)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH_3)(CH_3)-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH_2-N(OCH_3)-2' (and analogs thereof see published International Application WO/2008/150729, published

December 11, 2008); 4'-CH_2-O-N(CH_3)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-C(H)(CH_3)-2' (see Chattopadhyaya *et al., J. Org. Chem.,* 2009, *74*, 118-134); and 4'-CH_2-C(=CH_2)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

Further reports related to bicyclic nucleosides can also be found in published literature (see for example: Singh *et al., Chem. Commun.*, 1998, *4*, 455-456; Koshkin *et al., Tetrahedron*, 1998, *54*, 3607-3630; Wahlestedt *et al., Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; Kumar *et al., Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh *et al., J. Org. Chem.*, 1998, *63*, 10035-10039; Srivastava *et al., J. Am. Chem. Soc.*, 2007, *129(26)* 8362-8379; Elayadi *et al., Curr. Opinion Invest. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; and Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 7,034,133; 7,053,207; 7,399,845; 7,547,684; and 7,696,345; U.S. Patent Publication No. US2008-0039618; US2009-0012281; U.S. Patent Serial Nos. 60/989,574; 61/026,995; 61/026,998; 61/056,564; 61/086,231; 61/097,787; and 61/099,844; Published PCT International applications WO 1994/014226; WO 2004/106356; WO 2005/021570; WO 2007/134181; WO 2008/150729; WO 2008/154401; and WO 2009/006478. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

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

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl (S(=O)$_2$-J_1), or sulfoxyl (S(=O)-J_1); and

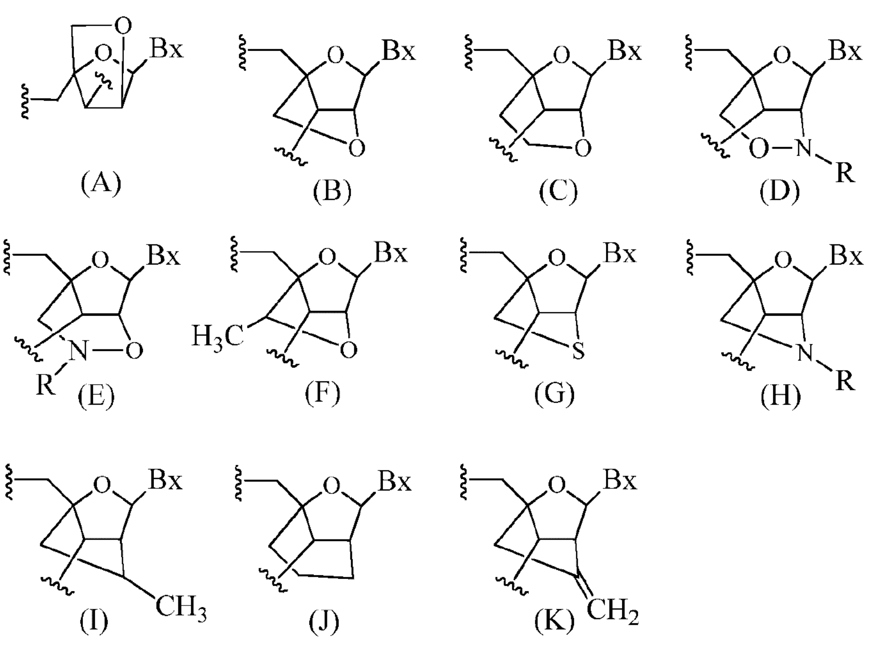
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-

H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl or a protecting group.

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

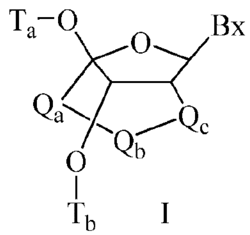
In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH_2-O-2') BNA's have been incorporated into antisense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

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



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

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



I

wherein:

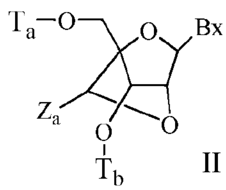
Bx is a heterocyclic base moiety;

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

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

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

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



II

wherein:

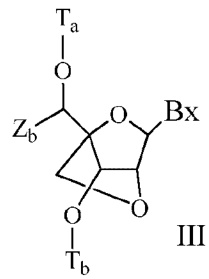
Bx is a heterocyclic base moiety;

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

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

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

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



III

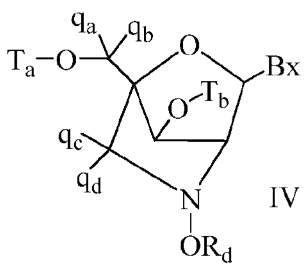
wherein:

Bx is a heterocyclic base moiety;

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

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

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



IV

wherein:

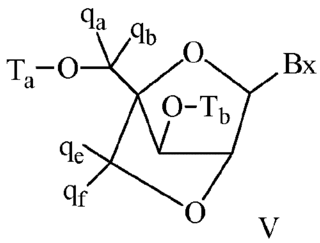
Bx is a heterocyclic base moiety;

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

R_d is C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

each q_a, q_b, q_c and q_d is, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl, C_1-C_6 alkoxyl, substituted C_1-C_6 alkoxyl, acyl, substituted acyl, C_1-C_6 aminoalkyl or substituted C_1-C_6 aminoalkyl;

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



V

wherein:

Bx is a heterocyclic base moiety;

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

q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O-C(=O)-NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

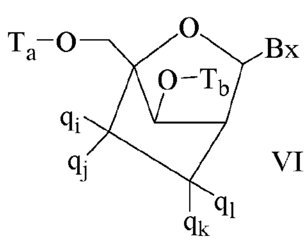
or q_e and q_f together are $=C(q_g)(q_h)$;

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

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

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

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



VI

wherein:

Bx is a heterocyclic base moiety;

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

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

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

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

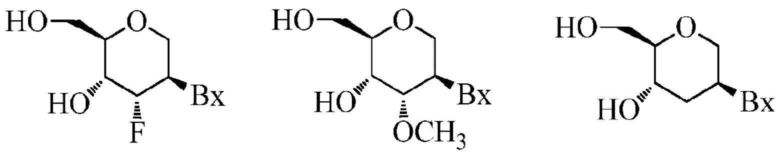
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

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

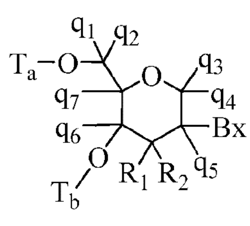
As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'-substituent groups can also be selected from: C_1-C_{12} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, F, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain

(Baker *et al.*, *J. Biol. Chem.*, 1997, *272*, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- *O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, *Helv. Chim. Acta*, 1995, *78*, 486-504; Altmann *et al.*, *Chimia*, 1996, *50*, 168-176; Altmann *et al.*, *Biochem. Soc. Trans.*, 1996, *24*, 630-637; and Altmann *et al.*, *Nucleosides Nucleotides*, 1997, *16*, 917-926).

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



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



VII

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

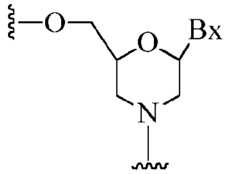
Bx is a heterocyclic base moiety;

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

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

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

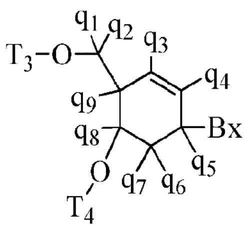
In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example nucleosides comprising morpholino sugar moieties and their use in oligomeric compounds has been reported (see for example: Braasch *et al.*, *Biochemistry*, 2002, *41*, 4503-4510;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



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

Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH_2-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.*, Srivastava *et al., J. Am. Chem. Soc.* 2007, *129(26)*, 8362-8379).

In certain embodiments, antisense compounds comprise one or more modified cyclohexenyl nucleosides, which is a nucleoside having a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Modified cyclohexenyl nucleosides include, but are not limited to those described in the art (see for example commonly owned, published PCT Application WO 2010/036696, published on April 10, 2010, Robeyns *et al., J. Am. Chem. Soc.*, 2008, *130(6)*, 1979-1984; Horváth *et al., Tetrahedron Letters,* 2007, *48*, 3621-3623; Nauwelaerts *et al., J. Am. Chem. Soc.*, 2007, *129(30)*, 9340-9348; Gu *et al.,, Nucleosides, Nucleotides & Nucleic Acids*, 2005, *24(5-7)*, 993-998; Nauwelaerts *et al., Nucleic Acids Research,* 2005, *33(8)*, 2452-2463; Robeyns et al., *Acta Crystallographica, Section F: Structural Biology and Crystallization Communications,* 2005, *F61(6)*, 585-586; Gu *et al., Tetrahedron*, 2004, *60(9)*, 2111-2123; Gu *et al., Oligonucleotides*, 2003, *13(6)*, 479-489; Wang *et al., J. Org. Chem.*, 2003, *68*, 4499-4505; Verbeure *et al., Nucleic Acids Research*, 2001, *29(24)*, 4941-4947; Wang *et al., J. Org. Chem.*, 2001, *66*, 8478-82; Wang *et al.*, *Nucleosides, Nucleotides & Nucleic Acids*, 2001, *20(4-7)*, 785-788; Wang *et al., J. Am. Chem.*, 2000, *122*, 8595-8602; Published PCT application, WO 06/047842; and Published PCT Application WO 01/049687). Certain modified cyclohexenyl nucleosides have Formula X.



X

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

Bx is a heterocyclic base moiety;

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

q_1, q_2, q_3, q_4, q_5, q_6, q_7, q_8 and q_9 are each, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl or other sugar substituent group.

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

As used herein, “2’-F” refers to a nucleoside comprising a sugar comprising a fluoro group at the 2’ position of the sugar ring.

As used herein, “2’-OMe” or “2’-OCH_3” or “2’-O-methyl” each refers to a nucleoside comprising a sugar comprising an -OCH_3 group at the 2’ position of the sugar ring.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

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

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

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

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

Modified Nucleobases

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

Additional modified nucleobases include 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-

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azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

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

In certain embodiments, antisense compounds targeted to a P23H rhodopsin nucleic acid comprise one or more modified nucleobases. In certain embodiments, shortened or gap-widened antisense oligonucleotides targeted to a P23H rhodopsin 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.

Conjugated Antisense compounds

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

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

In certain embodiments, antisense compounds, including, but not limited to those particularly suited for use as ssRNA, are modified by attachment of one or more conjugate groups. In general, conjugate groups modify one or more properties of the attached oligonucleotide, including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, cellular distribution, cellular uptake, charge and clearance. Conjugate groups are routinely used in the chemical arts and are linked directly or via an optional conjugate linking moiety or conjugate linking group to a parent compound such as an oligonucleotide. Conjugate groups includes without limitation, intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins and dyes. Certain conjugate groups have been described previously, for example: cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Let., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., do-decan-diol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

For additional conjugates including those useful for ssRNA and their placement within antisense compounds, see e.g., US Application No.; 61/583,963.

In vitro testing of antisense oligonucleotides

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

Cells may be treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

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

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

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

Yet another technique used to introduce antisense oligonucleotides into cultured cells includes free uptake of the oligonucleotides by the cells.

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

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

RNA Isolation

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

Compositions and Methods for Formulating Pharmaceutical Compositions

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

An antisense compound targeted to P23H rhodopsin nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutically acceptable diluent is water, such as sterile water suitable for injection. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to P23H rhodopsin nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is water. In certain embodiments, the antisense compound is an antisense oligonucleotide provided herein.

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

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

In certain embodiments, the compounds or compositions further comprise a pharmaceutically acceptable carrier or diluent.

EXAMPLES

The Examples below describe the screening process to identify lead compounds targeted to P23H mutant rhodopsin. Out of over 400 antisense oligonucleotides that were screened, ISIS 564426, ISIS 664844, ISIS 664867, and ISIS 664884 emerged as the top lead compounds. In particular, ISIS 664844 exhibited the best combination of properties in terms of potency, tolerability, and selectivity for P23H rhodopsin out of over 400 antisense oligonucleotides.

Non-limiting disclosure

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.

Although the sequence listing accompanying this filing identifies each sequence as either "RNA" or "DNA" as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

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Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligonucleotide having the nucleobase sequence "ATCGATCG" encompasses any oligonucleotides having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG".

Example 1: Design and in vitro screening of human rhodopsin

Antisense oligonucleotides were designed targeting human wild-type or P23H mutant rhodopsin nucleic acid and were tested for their effects on rhodopsin mRNA in vitro. Cell lines either expressing the entire rhodopsin genomic sequence or transfected with a mini gene were used in the assays. The cell lines are described further in the experiments in the Examples below. Two hundred and twelve MOE gapmers, with various motifs (5-10-5, 6-8-6, 7-6-7, 4-10-4, 5-8-5, 6-6-6, 3-10-3, 4-8-4, and 5-6-5) were tested in vitro for potency. Two hundred and two cEt gapmers, as well as gapmers with cEt and MOE modifications, were tested in vitro for potency. Of all these tested gapmers, 104 gapmers were tested in in vitro dose response assays.

The newly designed chimeric antisense oligonucleotides in the Table below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. 'Mismatch' indicates the number of mismatches the oligonucleotide sequence may have with the genomic sequence. Mismatches of more than 1 were not considered. The gapmers are targeted to the human rhodopsin genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_005612.16 truncated from nucleotides 35737800 to 35755500 or to the P23H rhodopsin mutant sequence having a cytosine to adenine substitution at position 163 of GENBANK Accession No. NM_000539.3; designated herein as SEQ ID NO:2 representing the mutant sequence), or both sequences. 'n/a' indicates that the particular oligonucleotide had more than one mismatch with the target gene sequence. The gapmers are presented in the Table below.

Table 1
3-10-3 gapmers targeting wild-type Rho (SEQ ID NO: 1) and P23H Rho (SEQ ID NO: 2)

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	Mismatch with SEQ ID NO: 1	Sequence	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
564387	4979	4994	1	AAGTGGCTGCGTACCA	151	166	11
564389	4983	4998	1	CTCGAAGTGGCTGCGT	155	170	12
564424	4977	4992	1	GTGGCTGCGTACCACA	149	164	13
564425	4981	4996	1	CGAAGTGGCTGCGTAC	153	168	14
564426	4985	5000	1	TACTCGAAGTGGCTGC	157	172	15
564283	4898	4913	0	CTTGTGGCTGACCCGT	70	85	65
564284	4935	4950	0	GAAGTTAGGGCCTTCT	107	122	66
564393	6112	6127	0	CAGCAGAGATATTCCT	n/a	n/a	67
564430	8414	8429	0	CAGGTAGGGAGACCCT	n/a	n/a	68
564433	8963	8978	0	CACCCGCAGTAGGCAC	n/a	n/a	69
564431	9444	9459	0	AGGAAATTGACTTGCC	n/a	n/a	70
564338	9851	9866	0	AGCAGAGGCCTCATCG	1085	1100	71
564342	9909	9924	0	GAGTCCTAGGCAGGTC	1143	1158	72
564299	10092	10107	0	GGTGGATGTCCCTTCT	1326	1341	73
564356	10192	10207	0	AAAGCAAGAATCCTCG	1426	1441	74
564307	10517	10532	0	GCTATTTACAAAGTGC	1751	1766	75
564370	10539	10554	0	ACTAGAATCTGTACAG	1773	1788	76
564372	10578	10593	0	ATTAACTAGTTACATT	1812	1827	77
564315	10654	10669	0	CCAAGGTTGGGTGAAA	1888	1903	78
564388	10757	10772	0	GGTCTGATGACTGCAT	1991	2006	79
564325	10791	10806	0	TTCACCGTCCCCCTCC	2025	2040	80
564329	10824	10839	0	AGGCCCAATCTCACCC	2058	2073	81
564399	10930	10945	0	AAGAGCAGGTGGCTTC	2164	2179	82
564349	11048	11063	0	CTAAGCTCTTCGAGAT	2282	2297	83
564363	11237	11252	0	AGCAGTTACTGAGGCA	2471	2486	84
564373	11359	11374	0	CAAAACCCACCACCGT	2593	2608	85
564381	11456	11471	0	TTGGCTCTGCTCATTG	2690	2705	86
564422	11465	11480	0	CTGTGCTGCTTGGCTC	2699	2714	87

Gapmers were tested at various doses in HEK-293 cells. HEK-293 cells expressing the the human genomic P23H rhodopsin sequence as a stable transfectant were used for these assays. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with antisense oligonucleotide, as specified in the Tables below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and rhodopsin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3374 (forward sequence GGAGGTCAACAACGAGTCTTTTG, designated herein as SEQ ID NO: 5; reverse sequence GGCCTCCTTGACGGTGAA, designated herein as SEQ ID NO: 6; probe sequence TTATCATCTTTTTCTGCTATGGGCAGCTCG, designated herein as SEQ ID NO: 7) was used to measure mRNA levels. Rhodopsin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of rhodopsin, relative to untreated control cells.

Table 2
Dose Response Inhibition of P23H RHO mRNA levels by 3-10-3 cEt gapmers targeted to SEQ ID NO: 2

ISIS No	1.25 µM	2.50 µM	5.00 µM	10.00 µM	20.00 µM	IC_{50} (µM)
564283	12	25	35	22	40	>20
564284	0	0	3	0	44	>20
564299	29	30	64	31	11	>20
564307	26	0	28	21	17	>20
564315	10	16	28	16	21	>20
564325	44	52	66	81	86	2
564329	0	2	10	16	0	>20
564349	0	0	0	0	1	>20
564363	17	0	20	13	31	>20
564373	19	17	10	29	38	>20
564381	16	18	34	33	42	>20
564387	19	26	39	42	76	7
564389	35	37	39	18	50	>20
564393	17	7	20	38	40	>20

Table 3
Dose Response Inhibition of P23H RHO mRNA levels by 3-10-3 cEt gapmers targeted to SEQ ID NO: 2

ISIS No	1.25 µM	2.50 µM	5.00 µM	10.00 µM	20.00 µM	IC_{50} (µM)
564338	0	20	35	19	25	>20
564342	32	31	40	0	36	>20
564356	21	18	31	13	0	>20
564370	0	0	15	10	17	>20
564372	0	0	0	0	23	>20
564388	0	0	20	27	2	>20
564399	9	0	24	30	35	>20

564422	4	0	20	17	51	9
564424	5	0	21	0	0	>20
564425	0	14	17	14	31	>20
564426	1	14	17	21	33	>20
564430	0	0	17	25	5	>20
564431	26	29	43	52	43	>20
564433	0	0	13	4	0	>20

Example 2: Design of antisense oligonucleotides with deoxy, 2'-alpha-fluoro, and cEt chemistry

Additional antisense oligonucleotides were designed with the same sequence as ISIS 564387 but with different chemistry. The new antisense oligonucleotides were designed as deoxy, 2'-alpha-fluoro and cEt oligonucleotides.

The 'Chemistry' column of the Table below presents chemical modifications in the oligonucleotide, including the position of the sugar modifications, wherein 'e' indicates a MOE modification, 'k' indicates a cEt modification, d indicates a deoxyribose sugar, and 'f' indicates a 2'-alpha-fluoro modification; 'mC' indicates 5-methycytosine; 'A', 'C', 'T', 'G', and 'U' represent the standard nucleotide notations. All the oligonucleotides are 15 or 16 nucleosides in length. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The antisense oligonucleotides were designed to target the mutant sequence (SEQ ID NO:2). The oligonucleotides are presented in the Table below. All the oligonucleotides target nucleotides 151-166 of SEQ ID NO: 2.

Table 4
Antisense oligonucleotides targeting the mutant P23H rhodopsin gene (SEQ ID NO: 2)

IsisNo	Chemistry	SEQ ID NO
564387	Aks Aks Gks Tds Gds Gds mCds Tds Gds mCds Gds Tds Ads mCks mCks Ak	11
598202	Aks Aks Gks Ufs Gds Gds mCds Tds Gds mCds Gds Tds Ads mCks mCks Ak	11
598203	Aks Aks Gks Tds Gfs Gds mCds Tds Gds mCds Gds Tds Ads mCks mCks Ak	11
598204	Aks Aks Gks Tds Gds Gfs mCds Tds Gds mCds Gds Tds Ads mCks mCks Ak	11
598205	Aks Aks Gks Tds Gds Gds Cfs Tds Gds mCds Gds Tds Ads mCks mCks Ak	11
598206	Aks Aks Gks Tds Gds Gds mCds Ufs Gds mCds Gds Tds Ads mCks mCks Ak	11
598207	Aks Aks Gks Tds Gds Gds mCds Tds Gfs mCds Gds Tds Ads mCks mCks Ak	11
598208	Aks Aks Gks Tds Gds Gds mCds Tds Gds Cfs Gds Tds Ads mCks mCks Ak	11
598209	Aks Aks Gks Tds Gds Gds mCds Tds Gds mCds Gfs Tds Ads mCks mCks Ak	11
598210	Aks Aks Gks Tds Gds Gds mCds Tds Gds mCds Gds Ufs Ads mCks mCks Ak	11
598211	Aks Aks Gks Tds Gds Gds mCds Tds Gds mCds Gds Tds Afs mCks mCks Ak	11

Example 3: Antisense inhibition of mutant P23H human rhodopsin

Additional antisense oligonucleotides were designed targeting the sequence region around the P23H mutation site of the rhodopsin gene and were tested for their effects on mutant rhodopsin mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HEK293 transfected with a SOD1 minigene containing mutant P23H rhodopsin were used in this assay.

The SOD1 minigene contains the unspliced sequence of SOD1 exon 4, intron 4, and exon 5, with a human rhodopsin sequence with the mutation at P23H. Each sequence was cloned into pcDNA4/TO at HindIII/EcoRI site.

HEK-293 cells with the SOD1 minigene containing mutant P23H rhodopsin were transfected using electroporation with 5μM or 20 μM antisense oligonucleotide. ISIS 564425, described in the study above, was also included in the assay. After a treatment period of approximately 24 hours, RNA was isolated from the cells and rhodopsin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS4220 (forward sequence CACTATAGGGAGACCCAAGC, designated herein as SEQ ID NO: 8; reverse sequence CTGCTTTTTCATGGACCACCA, designated herein as SEQ ID NO: 9; probe sequence CAAAGATGGTGTGGCCG, designated herein as SEQ ID NO: 10), which is targeted to the P23H site, was used to measure mRNA levels. Rhodopsin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of rhodopsin, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Table below were designed as 3-10-3 cEt gapmers, 3-9-3 cEt gapmers, deoxy, MOE and cEt oligonucleotides, or deoxy, 2'-alpha-fluoro and cEt oligonucleotides. The 'Chemistry' column of the Table below presents chemical modifications in the oligonucleotide, including the position of the sugar modifications, wherein 'e' indicates a MOE modification, 'k' indicates a cEt modification, d indicates a deoxyribose sugar, and 'f' indicates a 2'-alpha-fluoro modification; 'mC' indicates 5-methycytosine; 'A', 'C', 'T', 'G', and 'U' represent the standard nucleotide notations. All the oligonucleotides are 15 or 16 nucleosides in length. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The antisense oligonucleotides were designed to target the mutant P23H sequence (SEQ ID NO:2). The oligonucleotides are presented in the Table below.

Table 5
Inhibition of P23H rhodopsin mRNA by antisense oligonucleotides targeting the mutant rhodopsin gene (SEQ ID NO: 2)

ISIS NO	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Chemistry	Sequence	% inhibition (5 µM)	% inhibition (20 µM)	SEQ ID NO
564425	153	168	mCkGkAkAdGdTdGdGdmCdT dGdmCdGdTkAkmCk	CGAAGTGGCTGCGTAC	62	72	14
598206	151	166	AkAkGkTdGdGdmCdUfGdmCd GdTdAdmCkmCkAk	AAGTGGCUGCGTACCA	55	89	62
664823	149	164	GkTdGdGdmCdTdGdmCdGdTd AdmCkmCeAkmCeAk	GTGGCTGCGTACCACA	6	52	13
664824	150	165	AkGdTdGdGdmCdTdGdmCdG dTdAkmCemCkAemCk	AGTGGCTGCGTACCAC	50	77	16
664825	151	166	AkAdGdTdGdGdmCdTdGdmC dGdTkAemCkmCeAk	AAGTGGCTGCGTACCA	39	62	11
664826	152	167	GkAdAdGdTdGdGdmCdTdGd mCdGkTeAkmCemCk	GAAGTGGCTGCGTACC	46	66	17
664827	153	168	mCkGdAdAdGdTdGdGdmCdT dGdmCkGeTkAemCk	CGAAGTGGCTGCGTAC	53	52	14
664828	154	169	TkmCdGdAdAdGdTdGdGdmC dTdGkmCeGkTeAk	TCGAAGTGGCTGCGTA	40	66	18
664829	155	170	mCkTdmCdGdAdAdGdTdGdG dmCdTkGemCkGeTk	CTCGAAGTGGCTGCGT	35	59	12
664830	156	171	AkmCdTdmCdGdAdAdGdTdG dGdmCkTeGkmCeGk	ACTCGAAGTGGCTGCG	38	67	19
664831	157	172	TkAdmCdTdmCdGdAdAdGdTd GdGkmCeTkGemCk	TACTCGAAGTGGCTGC	39	63	15
664832	158	173	GkTdAdmCdTdmCdGdAdAdG dTdGkGemCkTeGk	GTACTCGAAGTGGCTG	10	51	20
664833	159	174	GkGdTdAdmCdTdmCdGdAdA dGdTkGeGkmCeTk	GGTACTCGAAGTGGCT	57	68	21
664834	149	164	GkTkGdGdmCdTdGdmCdGdTd AdmCdmCkAemCkAe	GTGGCTGCGTACCACA	33	50	13
664835	150	165	AkGkTdGdGdmCdTdGdmCdG dTdAdmCkmCeAkmCe	AGTGGCTGCGTACCAC	39	75	16
664836	151	166	AkAkGdTdGdGdmCdTdGdmC dGdTdAkmCemCkAe	AAGTGGCTGCGTACCA	56	76	11
664837	152	167	GkAkAdGdTdGdGdmCdTdGd mCdGdTkAemCkmCe	GAAGTGGCTGCGTACC	48	72	17
664838	153	168	mCkGkAdAdGdTdGdGdmCdT dGdmCdGkTeAkmCe	CGAAGTGGCTGCGTAC	38	84	14
664839	154	169	TkmCkGdAdAdGdTdGdGdmC dTdGdmCkGeTkAe	TCGAAGTGGCTGCGTA	49	72	18
664840	155	170	mCkTkmCdGdAdAdGdTdGdG dmCdTdGkmCeGkTe	CTCGAAGTGGCTGCGT	55	61	12
664841	156	171	AkmCkTdmCdGdAdAdGdTdG dGdmCdTkGemCkGe	ACTCGAAGTGGCTGCG	47	68	19
664842	157	172	TkAkmCdTdmCdGdAdAdGdTd GdGdmCkTeGkmCe	TACTCGAAGTGGCTGC	48	72	15
664843	158	173	GkTkAdmCdTdmCdGdAdAdG dTdGdGkmCeTkGe	GTACTCGAAGTGGCTG	64	73	20

664844	159	174	GkGkTdAdmCdTdmCdGdAdAdGdTdGkGemCkTe	GGTACTCGAAGTGGCT	61	64	21
664845	149	164	GkTkGdGdmCdTdGdmCdGdTdAdmCkmCeAkmCeAk	GTGGCTGCGTACCACA	10	45	13
664846	150	165	AkGkTdGdGdmCdTdGdmCdGdTdAkmCemCkAemCk	AGTGGCTGCGTACCAC	58	69	16
664847	151	166	AkAkGdTdGdGdmCdTdGdmCdGdTkAemCkmCeAk	AAGTGGCTGCGTACCA	41	56	11
664848	152	167	GkAkAdGdTdGdGdmCdTdGdmCdGkTeAkmCemCk	GAAGTGGCTGCGTACC	49	66	17
664849	153	168	mCkGkAdAdGdTdGdGdmCdTdGdmCkGeTkAemCk	CGAAGTGGCTGCGTAC	57	72	14
664850	154	169	TkmCkGdAdAdGdTdGdGdmCdTdGkmCeGkTeAk	TCGAAGTGGCTGCGTA	42	65	18
664851	155	170	mCkTkmCdGdAdAdGdTdGdGdmCdTkGemCkGeTk	CTCGAAGTGGCTGCGT	20	59	12
664852	156	171	AkmCkTdmCdGdAdAdGdTdGdGdmCkTeGkmCeGk	ACTCGAAGTGGCTGCG	42	57	19
664853	157	172	TkAkmCdTdmCdGdAdAdGdTdGdGkmCeTkGemCk	TACTCGAAGTGGCTGC	43	67	15
664854	158	173	GkTkAdmCdTdmCdGdAdAdGdTdGkGemCkTeGk	GTACTCGAAGTGGCTG	34	55	20
664855	159	174	GkGkTdAdmCdTdmCdGdAdAdGdTkGeGkmCeTk	GGTACTCGAAGTGGCT	48	63	21
664856	149	164	GkTkGkGdmCdTdGdmCdGdTdAdmCdmCkAemCkAe	GTGGCTGCGTACCACA	20	43	13
664857	150	165	AkGkTkGdGdmCdTdGdmCdGdTdAdmCkmCeAkmCe	AGTGGCTGCGTACCAC	0	37	16
664858	151	166	AkAkGkTdGdGdmCdTdGdmCdGdTdAkmCemCkAe	AAGTGGCTGCGTACCA	52	81	11
664859	152	167	GkAkAkGdTdGdGdmCdTdGdmCdGdTkAemCkmCe	GAAGTGGCTGCGTACC	52	74	17
664860	153	168	mCkGkAkAdGdTdGdGdmCdTdGdmCdGkTeAkmCe	CGAAGTGGCTGCGTAC	56	74	14
664861	154	169	TkmCkGkAdAdGdTdGdGdmCdTdGdmCkGeTkAe	TCGAAGTGGCTGCGTA	33	58	18
664862	155	170	mCkTkmCkGdAdAdGdTdGdGdmCdTdGkmCeGkTe	CTCGAAGTGGCTGCGT	39	64	12
664863	156	171	AkmCkTkmCdGdAdAdGdTdGdGdmCdTkGemCkGe	ACTCGAAGTGGCTGCG	45	70	19
664864	157	172	TkAkmCkTdmCdGdAdAdGdTdGdGdmCkTeGkmCe	TACTCGAAGTGGCTGC	49	69	15
664865	158	173	GkTkAkmCdTdmCdGdAdAdGdTdGdGkmCeTkGe	GTACTCGAAGTGGCTG	54	67	20
664866	159	174	GkGkTkAdmCdTdmCdGdAdAdGdTdGkGemCkTe	GGTACTCGAAGTGGCT	54	64	21
664867	157	172	TkAkmCkUfmCdGdAdAdGdTdGdGdmCdTkGkmCk	TACUCGAAGTGGCTGC	66	76	64
664868	157	172	TkAkmCkTdCfGdAdAdGdTdGdGdmCdTkGkmCk	TACTCGAAGTGGCTGC	54	69	15
664869	157	172	TkAkmCkTdmCdGfAdAdGdTdGdGdmCdTkGkmCk	TACTCGAAGTGGCTGC	53	69	15
664870	157	172	TkAkmCkTdmCdGdAfAdGdTdGdGdmCdTkGkmCk	TACTCGAAGTGGCTGC	54	69	15
664871	157	172	TkAkmCkTdmCdGdAdAfGdTdGdGdmCdTkGkmCk	TACTCGAAGTGGCTGC	45	68	15

664872	157	172	TkAkmCkTdmCdGdAdAdGfTd GdGdmCdTkGkmCk	TACTCGAAGTGGCTGC	46	72	15
664873	157	172	TkAkmCkTdmCdGdAdAdGdUf GdGdmCdTkGkmCk	TACTCGAAGUGGCTGC	42	72	63
664874	157	172	TkAkmCkTdmCdGdAdAdGdTd GfGdmCdTkGkmCk	TACTCGAAGTGGCTGC	48	69	15
664875	157	172	TkAkmCkTdmCdGdAdAdGdTd GdGfmCdTkGkmCk	TACTCGAAGTGGCTGC	44	66	15
664876	157	172	TkAkmCkTdmCdGdAdAdGdTd GdGdCfTkGkmCk	TACTCGAAGTGGCTGC	69	77	15
664877	150	164	GkTkGdGdmCdTdGdmCdGdTd AdmCdmCkAemCk	GTGGCTGCGTACCAC	9	43	22
664878	151	165	AkGkTdGdGdmCdTdGdmCdG dTdAdmCkmCeAk	AGTGGCTGCGTACCA	45	82	23
664879	152	166	AkAkGdTdGdGdmCdTdGdmC dGdTdAkmCemCk	AAGTGGCTGCGTACC	41	72	24
664880	153	167	GkAkAdGdTdGdGdmCdTdGd mCdGdTkAemCk	GAAGTGGCTGCGTAC	29	58	25
664881	154	168	mCkGkAdAdGdTdGdGdmCdT dGdmCdGkTeAk	CGAAGTGGCTGCGTA	35	63	26
664882	155	169	TkmCkGdAdAdGdTdGdGdmC dTdGdmCkGeTk	TCGAAGTGGCTGCGT	40	63	27
664883	156	170	mCkTkmCdGdAdAdGdTdGdG dmCdTdGkmCeGk	CTCGAAGTGGCTGCG	21	67	28
664884	157	171	AkmCkTdmCdGdAdAdGdTdG dGdmCdTkGemCk	ACTCGAAGTGGCTGC	53	78	29
664885	158	172	TkAkmCdTdmCdGdAdAdGdTd GdGdmCkTeGk	TACTCGAAGTGGCTG	49	78	30
664886	159	173	GkTkAdmCdTdmCdGdAdAdG dTdGdGkmCeTk	GTACTCGAAGTGGCT	51	64	31
664887	160	174	GkGkTdAdmCdTdmCdGdAdA dGdTdGkGemCk	GGTACTCGAAGTGGC	64	76	32
664899	150	164	GkTkGkGdmCdTdGdmCdGdTd AdmCdmCkAkmCk	GTGGCTGCGTACCAC	0	13	22
664900	151	165	AkGkTkGdGdmCdTdGdmCdG dTdAdmCkmCkAk	AGTGGCTGCGTACCA	52	81	23
664901	152	166	AkAkGkTdGdGdmCdTdGdmC dGdTdAkmCkmCk	AAGTGGCTGCGTACC	52	84	24
664902	153	167	GkAkAkGdTdGdGdmCdTdGd mCdGdTkAkmCk	GAAGTGGCTGCGTAC	41	77	25
664903	154	168	mCkGkAkAdGdTdGdGdmCdT dGdmCdGkTkAk	CGAAGTGGCTGCGTA	64	80	26
664904	155	169	TkmCkGkAdAdGdTdGdGdmC dTdGdmCkGkTk	TCGAAGTGGCTGCGT	43	45	27
664905	156	170	mCkTkmCkGdAdAdGdTdGdG dmCdTdGkmCkGk	CTCGAAGTGGCTGCG	48	68	28
664906	157	171	AkmCkTkmCdGdAdAdGdTdG dGdmCdTkGkmCk	ACTCGAAGTGGCTGC	59	77	29
664907	158	172	TkAkmCkTdmCdGdAdAdGdTd GdGdmCkTkGk	TACTCGAAGTGGCTG	51	71	30
664908	159	173	GkTkAkmCdTdmCdGdAdAdG dTdGdGkmCkTk	GTACTCGAAGTGGCT	55	67	31
664909	160	174	GkGkTkAdmCdTdmCdGdAdA dGdTdGkGkmCk	GGTACTCGAAGTGGC	65	69	32

Example 4: Potency and selectivity of antisense oligonucleotides targeting the mutant P23H rhodopsin gene

Antisense oligonucleotides from Example 3 exhibiting potent *in vitro* inhibition of the mutant P23H rhodopsin mRNA were selected and tested at various doses in HEK-293 cells transfected with either the mutant P23H (E5-M) or wild-type (E5-C) rhodopsin/SOD1 minigene construct.

The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cells were transfected using electroporation with 1.25 μM, 2.50 μM, 5.00 μM, 10.00 μM, and 20 μM concentrations of antisense oligonucleotide, as specified in the Tables below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and rhodopsin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS4220 was used to measure mRNA levels. Rhodopsin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of rhodopsin, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. Several antisense oligonucleotides selectively inhibited expression of the mutant P23H rhodopsin sequence compared to the WT sequence.

Table 6
Percent inhibition of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	1.250 μM	2.50 μM	5.00 μM	10.00 μM	20.00 μM	IC_{50} (μM)
598206	2	20	34	44	58	13
664833	0	8	0	24	18	>20
664836	0	13	7	29	39	>20
664843	0	2	14	20	13	>20
664844	0	2	12	16	6	>20
664846	0	8	14	33	52	19
664849	0	0	4	0	5	>20
664860	0	0	0	0	3	>20
664867	0	12	8	29	33	>20
664876	2	1	20	17	41	>20
664887	0	0	14	14	0	>20
664903	0	0	2	9	0	>20
664906	5	2	35	19	44	>20
664909	0	6	9	4	4	>20

Table 7
Percent inhibition of P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	1.250 μM	2.50 μM	5.00 μM	10.00 μM	20.00 μM	IC_{50} (μM)
598206	24	45	56	74	83	4
664833	11	37	49	60	66	6
664836	8	37	40	58	70	8
664843	40	42	48	62	61	5
664844	36	50	51	65	59	3
664846	0	17	31	45	63	12
664849	21	41	58	49	60	9
664860	21	43	54	60	72	4
664867	40	47	52	61	69	3
664876	2	27	58	67	67	4
664887	49	51	60	66	68	2
664903	40	48	58	72	73	3
664906	32	46	47	61	67	5
664909	28	47	58	60	54	3

Table 8
Percent inhibition of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	1.250 μM	2.50 μM	5.00 μM	10.00 μM	20.00 μM	IC_{50} (μM)
598206	0	15	31	51	60	10
664824	1	12	25	38	47	>20
664835	0	2	13	24	52	19
664838	0	2	0	23	26	>20
664840	8	13	23	22	40	>20
664848	0	0	10	6	14	>20
664858	9	22	21	48	51	17
664878	5	1	20	33	60	16
664884	6	10	19	30	50	>20
664885	0	0	0	22	0	>20
664900	16	28	31	45	55	15
664901	13	11	26	45	56	14
664902	0	3	0	22	19	>20
664908	0	15	4	18	14	>20

Table 9
Percent inhibition of P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	1.250 µM	2.50 µM	5.00 µM	10.00 µM	20.00 µM	IC_{50} (µM)
598206	30	44	58	72	84	3
664824	21	36	45	59	62	7
664835	1	16	29	36	66	11
664838	6	27	33	47	63	11
664840	3	45	29	35	62	14
664848	10	16	35	51	59	11
664858	55	58	53	62	70	4
664878	6	32	47	51	72	7
664884	28	37	51	57	68	6
664885	6	10	20	51	69	11
664900	44	51	52	65	71	2
664901	42	50	53	68	70	3
664902	0	27	38	57	64	8
664908	30	45	49	57	58	6

Example 5: Characterization of potency and selectivity of human antisense compounds targeting mutant P23H rhodopsin

Several additional antisense oligonucleotides were designed to target the mutant P23H rhodopsin gene and were transfected into either mutant P23H rhodopsin (E5-M) or wild-type (E5-C) rhodopsin/SOD1 minigene HEK293 cells. The SOD1 minigene sequence contains the unspliced sequence of SOD1 exon 4, intron 4, and exon 5, with the human wild-type rhodopsin or a rhodopsin sequence with the mutation at P23H. Each sequence was cloned into pcDNA4/TO at HindIII/EcoRI site.

Study 1

The newly designed chimeric antisense oligonucleotides in the Table below were designed as deoxy, MOE and cEt oligonucleotides with a 7 or 8 base deoxy gap Antisense oligonucleotides having a 7 or 8 base deoxy gap are potent and selective for targeting the SNP mutation of the hungtingtin (HTT) gene. Ostergaard ME et al., *Nucleic Acids Res.* 2013 Nov;41(21):9634-50; PCT Publication WO 2013/022990. It was expected that antisense oligonucleotides having a 7 or 8 base deoxy gap likewise would potently and selectively target P23H rhodopsin.

The 'Chemistry' column of the Table below presents chemical modifications in the oligonucleotide, including the position of the sugar modifications, wherein 'e' indicates a MOE modification, 'k' indicates a cEt modification, and the number indicates the number of deoxyribose sugars. All the oligonucleotides are 16 nucleosides in length. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosines are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The antisense oligonucleotides were designed to target the human mutant P23H rhodopsin sequence (SEQ ID NO:2). The oligonucleotides are presented in the Table below.

Table 10
Antisense oligonucleotides targeting mutant P23H rhodopsin (SEQ ID NO: 2)

ISIS No	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	Chemistry	SEQ ID NO
589177	148	163	TGGCTGCGTACCACAC	eekk-8-kkee	33
589193	148	163	TGGCTGCGTACCACAC	eeekk-7-kkee	33
589178	149	164	GTGGCTGCGTACCACA	eekk-8-kkee	13
589194	149	164	GTGGCTGCGTACCACA	eeekk-7-kkee	13
589179	150	165	AGTGGCTGCGTACCAC	eekk-8-kkee	16
589195	150	165	AGTGGCTGCGTACCAC	eeekk-7-kkee	16
589180	151	166	AAGTGGCTGCGTACCA	eekk-8-kkee	11
589196	151	166	AAGTGGCTGCGTACCA	eeekk-7-kkee	11
589181	152	167	GAAGTGGCTGCGTACC	eekk-8-kkee	17
589197	152	167	GAAGTGGCTGCGTACC	eeekk-7-kkee	17
589182	153	168	CGAAGTGGCTGCGTAC	eekk-8-kkee	14
589198	153	168	CGAAGTGGCTGCGTAC	eeekk-7-kkee	14
589183	154	169	TCGAAGTGGCTGCGTA	eekk-8-kkee	18
589199	154	169	TCGAAGTGGCTGCGTA	eeekk-7kkee	18
589184	155	170	CTCGAAGTGGCTGCGT	eekk-8-kkee	12
589200	155	170	CTCGAAGTGGCTGCGT	eeekk-7-kkee	12
589185	156	171	ACTCGAAGTGGCTGCG	eekk-8-kkee	19
589201	156	171	ACTCGAAGTGGCTGCG	eeekk-7-kkee	19
589186	157	172	TACTCGAAGTGGCTGC	eekk-8-kkee	15
589202	157	172	TACTCGAAGTGGCTGC	eeekk-7-kkee	15
589187	158	173	GTACTCGAAGTGGCTG	eekk-8-kkee	20
589203	158	173	GTACTCGAAGTGGCTG	eeekk-7-kkee	20
589188	159	174	GGTACTCGAAGTGGCT	eekk-8-kkee	21
589204	159	174	GGTACTCGAAGTGGCT	eeekk-7-kkee	21
589189	160	175	GGGTACTCGAAGTGGC	eekk-8-kkee	34
589205	160	175	GGGTACTCGAAGTGGC	eeekk-7-kkee	34
589190	161	176	TGGGTACTCGAAGTGG	eekk-8-kkee	35
589206	161	176	TGGGTACTCGAAGTGG	eeekk-7-kkee	35
589191	162	177	GTGGGTACTCGAAGTG	eekk-8-kkee	36

589207	162	177	GTGGGTACTCGAAGTG	eeekk-7-kkee	36
589192	163	178	TGTGGGTACTCGAAGT	eekk-8-kkee	37
589208	163	178	TGTGGGTACTCGAAGT	eeekk-7-kkee	37

The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. ISIS 564387 and ISIS 598206, described in the studies above, were also included in these assays. Cultured cells at a density of 20,000 cells per well were transfected using electroporation with antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and rhodopsin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS4220 was used to measure mRNA levels. Rhodopsin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of rhodopsin, relative to untreated control cells. A zero value only indicates that the antisense oligonucleotide did not inhibit mRNA expression.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. Some antisense oligonucleotides selectively reduced mutant P23H rhodopsin mRNA levels compared to WT rhodopsin expression.

Table 11

Percent reduction of mutant P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	740.7 nM	2222.2 nM	6666.7 nM	20000.0 nM	IC_{50} (μM)
564387	34	48	70	83	2
589177	22	29	47	65	8
589178	18	8	7	27	>20
589179	10	16	16	33	>20
589180	21	35	56	73	5
589181	20	22	50	67	8
589182	31	40	59	72	4
589183	17	44	47	64	7
589184	27	25	40	60	11
589185	1	30	37	61	11
589186	21	34	40	62	10
589187	28	37	59	64	5
589188	23	25	53	65	8
589189	16	19	48	56	11
589190	20	36	50	64	7
589191	0	20	40	49	17
589192	9	22	39	54	15
598206	41	54	72	84	1

Table 12
Percent reduction of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	740.7 nM	2222.2 nM	6666.7 nM	20000.0 nM	IC_{50} (μM)
564387	2	24	40	70	9
589177	14	27	31	64	13
589178	0	5	0	24	>20
589179	0	16	4	31	>20
589180	18	19	30	48	>20
589181	0	9	15	33	>20
589182	0	10	12	15	>20
589183	0	14	0	9	>20
589184	5	0	0	16	>20
589185	3	5	6	3	>20
589186	1	15	24	30	>20
589187	13	7	21	28	>20
589188	6	9	12	28	>20
589189	15	5	18	38	>20
589190	8	3	5	32	>20
589191	4	7	14	20	>20
589192	0	0	2	34	>20
598206	26	18	41	59	12

Table 13
Percent reduction of mutant P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	740.7 nM	2222.2 nM	6666.7 nM	20000.0 nM	IC_{50} (μM)
564387	0	51	67	82	2
589193	10	12	28	40	>20
589194	0	11	19	14	>20
589195	5	18	20	27	>20
589196	4	20	39	44	>20
589197	16	18	47	44	>20
589198	13	28	38	52	17
589199	12	18	31	36	>20
589200	2	11	32	52	20
589201	18	23	21	42	>20
589202	10	11	20	29	>20
589203	15	22	36	45	>20
589204	24	29	33	52	18
589205	5	19	27	40	>20
589206	6	9	22	39	>20

589207	4	11	25	51	20
589208	0	10	10	23	>20
598206	33	53	73	83	2

Table 14
Percent reduction of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	740.7 nM	2222.2 nM	6666.7 nM	20000.0 nM	IC_{50} (μM)
564387	0	22	40	60	11
589193	0	2	5	38	>20
589194	0	0	8	13	>20
589195	0	4	9	13	>20
589196	12	0	12	30	>20
589197	14	2	13	20	>20
589198	10	0	18	10	>20
589199	2	0	5	0	>20
589200	0	0	0	20	>20
589201	0	0	0	16	>20
589202	0	18	0	7	>20
589203	10	6	22	28	>20
589204	0	1	10	17	>20
589205	4	3	4	11	>20
589206	0	0	3	20	>20
589207	0	0	0	24	>20
589208	2	0	4	14	>20
598206	9	8	37	51	17

The summary table is shown below and indicates that only some, much fewer than expected, antisense oligonucleotides having a 7 or 8 base deoxy gap potently and selectively reduced mutant P23H rhodopsin mRNA levels compared to WT levels. The data show that the 7 or 8 base deoxy gap motif may not always be effective to potently and selectively target a mutation from one gene to another.

Table 15
Selectivity of antisense oligonucleotides

ISIS No	IC_{50} (µM) in WT Rho cells	IC_{50} (µM) in P23H Rho cells
564387	11	2
589177	13	8
589178	>20	>20
589179	>20	>20
589180	>20	5
589181	>20	8
589182	>20	4
589183	>20	7
589184	>20	11
589185	>20	11
589186	>20	10
589187	>20	5
589188	>20	8
589189	>20	11
589190	>20	7
589191	>20	17
589192	>20	15
589193	>20	>20
589194	>20	>20
589195	>20	>20
589196	>20	>20
589197	>20	>20
589198	>20	17
589199	>20	>20
589200	>20	20
589201	>20	>20
589202	>20	>20
589203	>20	>20
589204	>20	18
589205	>20	>20
589206	>20	>20
589207	>20	20
589208	>20	>20
598206	17	2

Study 2

Antisense oligonucleotides described in the studies above were further tested. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. ISIS 549144 (GGCCAATACGCCGTCA; designated herein as SEQ ID NO: 89), a 3-10-3 cEt gapmer that does not target any known gene, was used as a control. The results for each experiment are presented in separate tables shown below. Cultured HEK293 cells at a density of 30,000 cells per well were transfected using electroporation with antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and rhodopsin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS4220, which is targeted to the SOD1 mini gene, was used to measure mRNA levels. Rhodopsin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of rhodopsin, relative to untreated control cells. A zero value only indicates that the antisense oligonucleotide did not inhibit mRNA expression.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. Several antisense oligonucleotides reduced mutant rhodopsin mRNA levels potently and selectively.

Table 16
Percent reduction of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	IC_{50} (µM)
549144	0	7	0	0	0	>20
598206	2	20	34	44	58	13
664833	0	8	0	24	18	>20
664836	0	13	7	29	39	>20
664843	0	2	14	20	13	>20
664844	0	2	12	16	6	>20
664846	0	8	14	33	52	19
664849	0	0	4	0	5	>20
664860	0	0	0	0	3	>20
664867	0	12	8	29	33	>20
664876	2	1	20	17	41	>20
664887	0	0	14	14	0	>20
664903	0	0	2	9	0	>20
664906	5	2	35	19	44	>20
664909	0	6	9	4	4	>20

Table 17
Percent reduction of P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	IC_{50} (µM)
549144	0	0	0	2	0	>20
598206	24	45	56	74	83	4
664833	11	37	49	60	66	6
664836	8	37	40	58	70	8
664843	40	42	48	62	61	5
664844	36	50	51	65	59	3
664846	0	17	31	45	63	12
664849	21	41	58	49	60	9
664860	21	43	54	60	72	4
664867	40	47	52	61	69	3
664876	2	27	58	67	67	4
664887	49	51	60	66	68	2
664903	40	48	58	72	73	3
664906	32	46	47	61	67	5
664909	28	47	58	60	54	3

Table 18
Percent reduction of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	IC_{50} (µM)
549144	0	0	0	0	0	>20
598206	0	15	31	51	60	10
664824	1	12	25	38	47	>20
664835	0	2	13	24	52	19
664838	0	2	0	23	26	>20
664840	8	13	23	22	40	>20
664848	0	0	10	6	14	>20
664858	9	22	21	48	51	17
664878	5	1	20	33	60	16
664884	6	10	19	30	50	>20
664885	0	0	0	22	0	>20
664900	16	28	31	45	55	15
664901	13	11	26	45	56	14
664902	0	3	0	22	19	>20
664908	0	15	4	18	14	>20

Table 19
Percent reduction of P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	IC_{50} (µM)
549144	0	0	0	0	0	>20
598206	30	44	58	72	84	3
664824	21	36	45	59	62	7
664835	1	16	29	36	66	11
664838	6	27	33	47	63	11
664840	3	45	29	35	62	14
664848	10	16	35	51	59	11
664858	55	58	53	62	70	4
664878	6	32	47	51	72	7
664884	28	37	51	57	68	6
664885	6	10	20	51	69	11
664900	44	51	52	65	71	2
664901	42	50	53	68	70	3
664902	0	27	38	57	64	8
664908	30	45	49	57	58	6

The summary table is shown below and indicates that some antisense oligonucleotides, including ISIS 664844, potently and selectively reduced mutant rhodopsin mRNA levels compared to WT rhodopsin levels.

Table 20
Selectivity of antisense oligonucleotides

ISIS No	IC_{50} (µM) in WT Rho cells	IC_{50} (µM) in P23H Rho cells
549144	>20	>20
598206	10	3
664824	>20	7
664833	>20	6
664835	19	11
664836	>20	8
664838	>20	11
664840	>20	14
664843	>20	5
664844	>20	3
664846	19	12

664848	>20	11
664849	>20	9
664858	17	4
664860	>20	4
664867	>20	3
664876	>20	4
664878	16	7
664884	>20	6
664885	>20	11
664887	>20	2
664900	15	2
664901	14	3
664902	>20	8
664903	>20	3
664906	>20	5
664908	>20	6
664909	>20	3

Study 3

Antisense oligonucleotides from the studies described above were further tested. Two new oligonucleotides were designed and are presented in the Table below.

ISIS 586139 is a 3-10-3 cEt gapmer, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. ISIS 643801 is a 2-10-2 cEt gapmer, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising two nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The antisense oligonucleotides were designed to target the mutant sequence (SEQ ID NO:2). The oligonucleotides are presented in the Table below.

Table 21
Antisense oligonucleotides targeting P23H rhodopsin (SEQ ID NO: 2)

ISIS No	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	SEQ ID NO
586139	158	173	GTACTCGAAGTGGCTG	20
643801	152	165	AGTGGCTGCGTACC	38

The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. ISIS 549144 was used as a control. The results for each experiment are presented in separate tables shown below. Cultured HEK293 cells having the SOD-1 minigene at a density of 30,000 cells per well were transfected using electroporation with antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and rhodopsin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS4220, which is targeted to the SOD1 mini gene, was used to measure mRNA levels. Rhodopsin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of rhodopsin, relative to untreated control cells. A zero value only indicates that the antisense oligonucleotide did not inhibit mRNA expression.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. Several antisense oligonucleotides reduced mutant rhodopsin mRNA levels potently and selectively.

Table 22
Percent reduction of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	0.5 µM	1.5 µM	4.4 µM	13.3 µM	40 µM	IC_{50} (µM)
549144	0	1	0	4	0	>40
564387	0	22	42	59	78	8
564389	0	0	0	33	38	>40
564425	2	0	5	17	7	>40
564426	10	19	35	45	61	17
564431	0	0	0	0	4	>40
586139	3	20	15	35	53	33
589177	37	54	53	62	64	3
643801	0	12	27	53	68	14
664838	0	0	0	12	16	>40
664843	0	25	13	41	50	34
664844	0	3	6	10	17	>40

664860	0	0	0	9	10	>40
664867	0	16	4	44	52	29
664884	3	0	0	43	53	28
664885	0	0	0	3	13	>40

Table 23
Percent reduction of P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	0.5 µM	1.5 µM	4.4 µM	13.3 µM	40 µM	IC_{50} (µM)
549144	0	0	4	0	10	>40
564387	8	36	69	85	90	3
564389	0	37	64	77	77	3
564425	25	41	47	75	80	3
564426	26	43	49	79	80	2
564431	0	0	0	10	17	>40
586139	34	42	63	75	83	2
589177	28	33	40	60	64	8
643801	1	27	49	72	88	5
664838	0	16	39	45	78	9
664843	23	31	64	76	78	3
664844	29	56	66	75	73	2
664860	11	36	65	77	83	3
664867	17	44	64	76	82	3
664884	0	28	54	71	84	5
664885	0	25	53	73	83	5

The results of studies in mutant and WT cells are summarized in the Table below. The IC_{50} values show the potency of certain oligonucleotides. The data shows that some oligonucleotides, including ISIS 664844, demonstrate potency and selectivity for the human mutant P23H rhodopsin gene. The sequence of the oligonucleotide with the mutation bolded and underlined is also shown.

Table 24
IC_{50} for the WT and P23H mutant cells

ISIS No	Sequence with mutation	WT	Mutant	SEQ ID NO:
564389	CTCGAAG**T**GGCTGCGT	>40	3	12
564426	TACTCGAAG**T**GGCTGC	17	2	15
664844	GGTACTCGAAG**T**GGCT	>40	2	21
664860	CGAAG**T**GGCTGCGTAC	>40	3	14
664867	TACUCGAAG**T**GGCTGC	29	3	64
664884	ACTCGAAG**T**GGCTGC	28	5	29

Example 6: Efficacy of antisense oligonucleotides targeting human rhodopsin in transgenic mice

Additional antisense oligonucleotides were designed and tested in two transgenic (Tg) mice models. The germline of these mice were inserted with a P23H mutant allele from a retinitis pigmentosa patient (Olsson, J.E. et al., Neuron. 1992. 9: 815-830). A total of 144 antisense oligonucleotides were tested. Not all the antisense oligonucleotides tested demonstrated potency in inhibiting mutant rhodopsin expression.

Study 1

P23HTg mice were treated with ISIS oligonucleotides described in the studies above. Two newly designed 3-10-3 cEt gapmers targeted to rhodopsin away from the P23H site, ISIS 564426 and ISIS 564432, were also included in the study.

Table 25

3-10-3 cEt gapmers targeting human rhodopsin (SEQ ID NO: 1)

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	Sequence	SEQ ID NO
564429	7798	7813	TAAGAAATGGACCCTA	39
564432	8692	8707	CCCGGGTCCAGACCAT	40

P23H Tg mice were randomly divided into treatment groups of 3-5 mice each. The gapmers were injected at a dose of 50 µg via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human P23H rhodopsin expression from eye tissue was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in mice treated with PBS. The data are presented in the Table below and demonstrate that some antisense oligonucleotides reduced mutant human P23H rhodopsin expression in vivo.

Table 26

% inhibition of human P23H rhodopsin mRNA

ISIS No	%
564431	89
564299	41
564329	38
564363	21

564370	34
564372	15
564373	33
564422	43
564429	31
564432	6
564433	7

Study 2

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers, 5-7-4 cEt gapmers, 5-10-5 MOE gapmers, 6-8-6 MOE gapmers, 7-6-7 MOE gapmers, 4-10-4 MOE gapmers, 5-8-5 MOE gapmers, 4-8-4 MOE gapmers, or 5-6-5 MOE gapmers.

The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The 5-7-4 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises seven 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five and four nucleosides respectively. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 6-8-6 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 7-6-7 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises six 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising seven nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 4-10-4 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising four nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 5-8-5 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 4-8-4 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising four nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 5-6-5 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises six 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification.

The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The antisense oligonucleotides were designed to target the mutant sequence (SEQ ID NO:2).

P23H Tg mice were randomly divided into treatment groups of 3-5 mice each. The gapmers were injected at a dose of 50 μg via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in mice treated with PBS. A '0' value inhibition only indicates that the oligonucleotide did not inhibit expression of in this particular instance. The data are presented in the Table below.

Table 27
Inhibition of rhodopsin expression in P23H Tg mice

ISIS NO	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Motif	Sequence	% inhibition	SEQ ID NO
564426	157	172	3-10-3 cEt	TACTCGAAGTGGCTGC	41	15
598213	152	167	5-7-4 cEt	GAAGTGGCTGCGTACC	0	17
614060	150	169	5-10-5 MOE	TCGAAGTGGCTGCGTACCAC	6	41
614067	157	176	5-10-5 MOE	TGGGTACTCGAAGTGGCTGC	0	42
614068	158	177	5-10-5 MOE	GTGGGTACTCGAAGTGGCTG	0	43
614074	164	183	5-10-5 MOE	AGTACTGTGGGTACTCGAAG	0	44
614075	143	162	6-8-6 MOE	GGCTGCGTACCACACCCGTC	9	45
614082	150	169	6-8-6 MOE	TCGAAGTGGCTGCGTACCAC	7	41
614083	151	170	6-8-6 MOE	CTCGAAGTGGCTGCGTACCA	0	46
614089	157	176	6-8-6 MOE	TGGGTACTCGAAGTGGCTGC	0	42
614105	151	170	7-6-7 MOE	CTCGAAGTGGCTGCGTACCA	0	46
614111	157	176	7-6-7 MOE	TGGGTACTCGAAGTGGCTGC	0	42

614166	150	167	4-10-4 MOE	GAAGTGGCTGCGTACCAC	0	47
614167	151	168	4-10-4 MOE	CGAAGTGGCTGCGTACCA	34	48
614187	151	168	5-8-5 MOE	CGAAGTGGCTGCGTACCA	0	48
614188	152	169	5-8-5 MOE	TCGAAGTGGCTGCGTACC	1	49
614194	158	175	5-8-5 MOE	GGGTACTCGAAGTGGCTG	0	50
614195	159	176	5-8-5 MOE	TGGGTACTCGAAGTGGCT	11	51
614250	158	173	4-8-4 MOE	GTACTCGAAGTGGCTG	5	20
614251	159	174	4-8-4 MOE	GGTACTCGAAGTGGCT	0	21
614263	153	168	5-6-5 MOE	CGAAGTGGCTGCGTAC	0	14
614268	158	173	5-6-5 MOE	GTACTCGAAGTGGCTG	16	20

Study 3

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers, 6-8-6 MOE gapmers, 4-10-4 MOE gapmers, 5-8-5 MOE gapmers, 6-6-6 MOE gapmers, 3-10-3 MOE gapmers, or 4-8-4 MOE gapmers.

The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The 6-8-6 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 4-10-4 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising four nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 5-8-5 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 6-6-6 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises six 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 3-10-3 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The 4-8-4 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising four nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The antisense oligonucleotides were designed to target the mutant sequence (SEQ ID NO:2).

P23H Tg mice were randomly divided into treatment groups of 3-5 mice each. The gapmers were injected at a dose of 50 μg via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in mice treated with PBS. A '0' value inhibition only indicates that the oligonucleotide did not inhibit expression of in this particular instance. The data are presented in the Table below.

Table 28
Inhibition of rhodopsin expression in P23H Tg mice

ISIS NO	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Motif	Sequence	% inhibition	SEQ ID NO
614225	151	166	3-10-3 MOE	AAGTGGCTGCGTACCA	1	11
614208	152	169	6-6-6 MOE	TCGAAGTGGCTGCGTACC	0	49
614226	152	167	3-10-3 MOE	GAAGTGGCTGCGTACC	3	17
614244	152	167	4-8-4 MOE	GAAGTGGCTGCGTACC	2	17
614227	153	168	3-10-3 MOE	CGAAGTGGCTGCGTAC	0	14
614245	153	168	4-8-4 MOE	CGAAGTGGCTGCGTAC	0	14
614246	154	169	4-8-4 MOE	TCGAAGTGGCTGCGTA	0	18
614088	156	175	6-8-6 MOE	GGGTACTCGAAGTGGCTGCG	3	52
614192	156	173	5-8-5 MOE	GTACTCGAAGTGGCTGCG	5	53
614193	157	174	5-8-5 MOE	GGTACTCGAAGTGGCTGC	6	54
614231	157	172	3-10-3 MOE	TACTCGAAGTGGCTGC	11	15
614232	158	173	3-10-3 MOE	GTACTCGAAGTGGCTG	10	20
614233	159	174	3-10-3 MOE	GGTACTCGAAGTGGCT	0	44
586141	160	175	3-10-3 cEt	GGGTACTCGAAGTGGC	0	34
586143	162	177	3-10-3 cEt	GTGGGTACTCGAAGTG	0	36

614178	162	179	4-10-4 MOE	CTGTGGGTACTCGAAGTG	30	55
564340	1133	1148	3-10-3 cEt	CAGGTCTTAGGCCGGG	20	56

Study 4

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers or deoxy, 2'-fluoro and cEt oligonucleotides. The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The deoxy, 2'-fluoro and cEt oligonucleotides are 16 nucleosides in length. The 'Chemistry' column of the Table below presents the position of the sugar modifications, wherein 'e' indicates a MOE modification, 'k' indicates a cEt modification, d indicates a deoxyribose sugar, and 'f' indicates a 2'-alpha-fluoro modification; 'mC' indicates 5-methycytosine; 'A', 'C', 'T', 'G', and 'U' represent the standard nucleotide notations. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The gapmers are targeted to either the human rhodopsin genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_005612.16 truncated from nucleotides 35737800 to 35755500) or the mutant sequence (SEQ ID NO:2), or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

P23H Tg mice were randomly divided into treatment groups of 3-5 mice each. ISIS 564340 from the studies described above was also included in this assay. 3-10-3 cEt gapmers were injected at a dose of 50 μg via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression from eye tissue was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in mice treated with PBS.

Table 29
Inhibition of rhodopsin expression in P23H Tg mice

ISIS NO	Motif	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
586138	AkmCkTkmCdGdAdAdGdTdGdGdmCdTdGkmCkGk	ACTCGAAGTGGCTGCG	24	156	171	19
598204	AkAkGkTdGdGfmCdTdGdmCdGdTdAdmCkmCkAk	AAGTGGCTGCGTACCA	2	151	166	11
598208	AkAkGkTdGdGdmCdTdGdCfGdTdAdmCkmCkAk	AAGTGGCTGCGTACCA	15	151	166	11

598211	AkAkGkTdGdGdmCdTdGdmCdGdTdAfmCkmCkAk	AAGTGGCTGCGTACCA	13	151	166	11
564340	mCkAkGkGdTdmCdTdTdAdGdGdmCdmCdGkGkGk	CAGGTCTTAGGCCGGG	21	1133	1148	56

Study 5

Additional oligonucleotides were designed with the same sequence as antisense oligonucleotides described above but with different chemistries. The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers or deoxy, 2'-fluoro and cEt oligonucleotides. The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The deoxy, 2'-fluoro and cEt oligonucleotides are 16 nucleosides in length. The 'Chemistry' column of the Table below presents the position of the sugar modifications, wherein 'e' indicates a MOE modification, 'k' indicates a cEt modification, d indicates a deoxyribose sugar, and 'f' indicates a 2'-alpha-fluoro modification; 'mC' indicates 5-methycytosine; 'A', 'C', 'T', 'G', and 'U' represent the standard nucleotide notations. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages.

'Parent oligo' indicates the ISIS oligonucleotide with the same sequence as the newly designed oligonucleotide. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The gapmers are targeted to the human mutant P23H sequence (SEQ ID NO:2). 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 30
Antisense oligonucleotides targeting SEQ ID NO: 2

ISIS NO	Parent oligo	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Motif	Sequence	SEQ ID NO
586136	-	152	167	GkAkAkGdTdGdGdmCdTdGdmCdGdTdAkmCkmCk	GAAGTGGCTGCGTACC	17
586137	-	154	169	TkmCkGkAdAdGdTdGdGdmCdTdGdmCdGkTkAk	TCGAAGTGGCTGCGTA	18
598212	586136	152	167	GkAkAkGkTdGdGdmCdTdGdmCdGdTkAkmCkmCk	GAAGTGGCTGCGTACC	17
598214	561125	153	168	mCkGkAkAkGdTdGdGdmCdTdGdmCdGkTkAkmCk	CGAAGTGGCTGCGTAC	14
598215	564425	153	168	mCkGkAkAkGkTdGdGdmCdTdGdmCdGkTkAkmCk	CGAAGTGGCTGCGTAC	14
598216	586137	154	169	TkmCkGkAkAdGdTdGdGdmCdTdGdmCkGkTkAk	TCGAAGTGGCTGCGTA	18
598217	586137	154	169	TkmCkGkAkAkGdTdGdGdmCdTdGdmCkGkTkAk	TCGAAGTGGCTGCGTA	18
598218	564389	155	170	mCkTkmCkGkAdAdGdTdGdGdmCdTdGkmCkGdTk	CTCGAAGTGGCTGCGT	12
598219	564389	155	170	mCkTkmCkGkAkAdGdTdGdGdmCdTdGkmCkGkTk	CTCGAAGTGGCTGCGT	12

P23H Tg mice were randomly divided into treatment groups of 3-5 mice each. ISIS 564431 and ISIS 598206, described in the studies above were also included in this assay. The antisense oligonucleotides were injected at a dose of 50 μg via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression from eye tissue was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in mice treated with PBS. A '0' value inhibition only indicates that the oligonucleotide did not inhibit expression of in this particular instance. The data are presented in the Table below and demonstrate that some antisense oligonucleotides reduced mutant human rhodopsin expression in vivo.

Table 31
Percent inhibition of mutant P23H rhodopsin expression

ISIS NO	% inhibition
564431	64
586136	29
586137	19
598206	51
598209	14
598210	10
598212	8
598214	47
598215	10
598216	25
598217	4
598218	20
598219	10

Example 7: Potency and selectivity of human antisense compounds targeting human mutant P23H rhodopsin

Additional antisense oligonucleotides were designed targeting the P23H site of human mutant P23H rhodopsin. These oligonucleotides as well as antisense oligonucleotides described in the studies above were further tested. The oligonucleotides were transfected into either HEK293 cells expressing either P23H mutant rhodopsin/SOD1 minigene (E5-M) or wild-type rhodopsin/SOD1 minigene (E5-C).

The new antisense oligonucleotides were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. The gapmers are targeted to the human mutant P23H sequence (SEQ ID NO:2. 'Mismatch indicates the number of mismatches the oligonucleotide has with the rhodopsin sequence in addition the to P23H mutation

Table 32
3-10-3 cEt gapmers targeted to SEQ ID NO: 2

ISIS No	Sequence	Start Site on SEQ ID NO: 2	Stop Site on SEQ ID NO: 2	Mismatches with SEQ ID NO: 2	SEQ ID NO
586125	GGGGCTGCGTACCACA	149	164	1	57
586126	AAGGGGCTGCGTACCA	151	166	1	58
586127	CGAAGGGGCTGCGTAC	153	168	1	59
586128	CTCGAAGGGGCTGCGT	155	170	1	60
586129	TACTCGAAGGGGCTGC	157	172	1	61

The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured cells at a density of 30,000 cells per well were transfected using electroporation with antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and rhodopsin mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3374 was used to measure mRNA levels. Rhodopsin mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of rhodopsin, relative to untreated control cells. A zero value only indicates that the antisense oligonucleotide did not inhibit mRNA expression.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. Several antisense oligonucleotides differentially reduced mutant rhodopsin mRNA levels compared to WT rhodopsin expression.

Table 33
Percent reduction of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	0.74 µM	2.22 µM	6.67 µM	20.00 µM	IC_{50} (µM)
564389	0	25	26	67	12
564425	0	16	31	55	16
586136	6	23	54	72	7
586137	0	18	28	58	15

598202	25	34	60	79	4
598203	10	26	43	69	8
598204	12	30	50	81	5
598205	0	21	39	66	10
598206	23	28	68	81	4
598207	0	15	53	70	8
598208	22	38	64	81	4
598209	0	18	50	75	7
598210	10	14	45	76	8
598211	14	39	69	80	4
598212	19	16	27	45	>20
598213	25	0	30	61	14
564325	17	22	35	53	17
564431	35	36	45	66	7
564387	18	35	53	53	6

Table 34
Percent reduction of P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	0.74 μM	2.22 μM	6.67 μM	20.00 μM	IC_{50} (μM)
564389	15	33	42	57	11
564425	0	39	49	58	8
586136	14	33	55	72	6
586137	10	39	26	62	13
598202	20	40	58	65	4
598203	0	20	46	57	11
598204	8	29	52	61	8
598205	1	24	38	59	12
598206	15	49	66	67	3
598207	16	29	49	54	11
598208	20	30	59	54	5
598209	17	33	53	63	7
598210	14	29	50	68	7
598211	17	39	58	77	4
598212	14	21	51	64	8
598213	8	13	27	42	>20
564325	31	18	29	56	17
564431	15	33	45	54	12
564387	24	32	51	51	12

Table 35
Percent reduction of wild-type rhodopsin mRNA in WT HEK293 cells (E5-C)

ISIS No	0.74 µM	2.22 µM	6.67 µM	20.00 µM	IC_{50} (µM)
598214	0	4	28	51	19
598215	0	9	17	41	>20
598216	0	3	16	48	>20
598217	0	6	10	30	>20
598218	0	8	18	25	>20
598219	0	9	7	29	>20
564389	13	0	36	63	14
564424	10	4	31	47	>20
564425	0	0	20	60	19
564426	0	16	47	56	11
586125	35	49	69	74	2
586126	18	27	57	71	6
586127	12	25	51	68	7
586128	14	37	50	65	7
586129	52	67	81	83	1
564325	25	28	36	61	12
564431	13	41	59	60	4
564387	7	12	54	76	7

Table 36
Percent reduction of P23H rhodopsin mRNA in mutant HEK293 cells (E5-M)

ISIS No	0.74 µM	2.22 µM	6.67 µM	20.00 µM	IC_{50} (µM)
598214	32	46	57	72	3
598215	21	39	39	66	8
598216	18	26	30	53	17
598217	7	20	16	50	20
598218	5	6	21	48	>20
598219	0	13	33	45	>20
564389	1	39	31	60	12
564424	0	24	25	44	>20
564425	20	41	51	54	9
564426	19	31	50	60	8
586125	0	19	25	53	20
586126	15	22	35	42	>20
586127	7	13	4	28	>20
586128	2	10	18	18	>20
586129	17	19	34	48	>20
564325	30	23	33	50	19

564431	2	24	39	42	>20
564387	11	12	53	64	9

The summary table is shown below and indicates that only a few antisense oligonucleotides selectively reduced mutant rhodopsin mRNA levels compared to WT rhodopsin levels. A selectivity of '1' indicates that the antisense oligonucleotide did not selectively reduce the mutant sequence compared to the control. A negative selectivity value indicates that the antisense oligonucleotide targeted the wild-type sequence more potently than the mutant sequence.

Table 37
Selectivity of antisense oligonucleotides

ISIS No	Selectivity
598214	5.8
598215	2.6
598216	1.2
598217	1.0
598218	1.0
598219	1.0
564389	1.0
564424	1.0
564425	1.7
564426	1.4
586125	-9.5
586126	-3.5
586127	-2.8
586128	-2.9
586129	-31.5
564325	-1.6
564431	-5.0
564387	-1.2
564389	1.0
564425	2.0
586136	1.2
586137	1.2
598202	1.0
598203	-1.4
598204	-1.5
598205	-1.2
598206	1.3
598207	1.5
598208	-1.3
598209	1.0

598210	1.2
598211	-1.1
598212	2.5
598213	-1.5
564325	1.0
564431	-1.7
564387	-2.1

Example 8: Efficacy and selectivity of antisense oligonucleotides targeting human rhodopsin in transgenic mice

Antisense oligonucleotides selected from the studies described above were further tested in transgenic mouse models. The germline of these mice were inserted with either a wild-type rhodopsin allele or a P23H mutant rhodopsin allele from a retinitis pigmentosa patient.

Study 1

P23H Tg mice were randomly divided into treatment groups of 4 mice each. ISIS oligonucleotides were injected via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression from eye tissue was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in the eye tissue treated with PBS. A '0' value inhibition only indicates that the oligonucleotide did not inhibit expression of in this particular instance. The data are presented in the Table below and demonstrated that the antisense oligonucleotides inhibit expression of mutant P23H rhodopsin gene in a dose-dependent manner.

Table 38
% inhibition of human mutant P23H rhodopsin expression

ISIS No	Chemistry	Dose (μg)	% inhibition
564431	3-10-3 cEt	20	64
564426	3-10-3 cEt	50	63
		20	42
		10	12
664844	Deoxy, MOE, and cEt	50	50
		20	41
		10	32

664860	Deoxy, MOE, and cEt	50	44
		20	39
		10	30
664867	Deoxy, 2'-alpha-fluoro and cEt	50	62
		20	25
		10	0
664884	Deoxy, MOE, and cEt	50	68
		20	48
		10	17

Study 2

Human WT rhodopsin Tg mice were randomly divided into treatment groups of 3-6 mice each. ISIS oligonucleotides, selected from the studies described above, were injected via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression from eye tissue was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in the eye tissue treated with PBS. A '0' value inhibition only indicates that the oligonucleotide did not inhibit expression of in this particular instance. The results are presented in the Table below and demonstrate the several antisense oligonucleotides do not effectively inhibit expression of the wild-type rhodopsin gene.

Table 39
% reduction in human WT rhodopsin expression

ISIS No	Chemistry	Dose (µg)	% inhibition
564389	3-10-3 cEt	50	10
		20	0
		10	10
564426	3-10-3 cEt	50	21
		20	3
		10	0
664844	Deoxy, MOE and cEt	50	22
		20	24
		10	0

664860	Deoxy, MOE and cEt	50	39
		20	19
		10	5
664884	Deoxy, MOE and cEt	50	28
		20	0
		10	2
664867	Deoxy, 2'-alpha-fluoro and cEt	50	9
		20	16
		10	7

Example 9: Confirmation of efficacy and selectivity of antisense oligonucleotides targeting human rhodopsin in transgenic mice

Select antisense oligonucleotides that demonstrated potency and selectivity in the studies described above were further tested in the human P23H or wild-type rhodopsin transgenic mouse models. The data demonstrates the selectivity of the leads for the mutant rhodopsin gene.

Study 1

P23H Tg mice were randomly divided into treatment groups of 4 mice each. ISIS oligonucleotides, selected from the studies described above, were injected via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression from eye tissue was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in the eye treated with PBS. The data presented in the Table below are the average of two separate experiments and demonstrate that the antisense oligonucleotides inhibit expression of mutant rhodopsin gene in a dose-dependent manner.

Table 40
% inhibition of human mutant P23H rhodopsin expression

ISIS No	Chemistry	Dose (μg)	% inhibition
564426	3-10-3 cEt	50	68
		35	45
		20	27
664844	Deoxy, MOE, and cEt	50	40
		35	37
		20	20

664867	Deoxy, 2'-alpha-fluoro and cEt	50	58
		35	43
		20	26
664884	Deoxy, MOE, and cEt	50	51
		35	48
		20	25

Study 2

Human WT rhodopsin Tg mice were randomly divided into treatment groups of 4 mice each. ISIS oligonucleotides, selected from the studies described above, were injected via intravitreal injection in the right eye of each of the mice. The left eye of the animals was injected with PBS and served as the control. Mice were sacrificed after 7 days. Human rhodopsin expression from eye tissue was measured with the human-specific primer probe set RTS3363. The results are normalized to the expression of mouse cone rod homeobox. Percent inhibition is relative to the expression seen in the eye treated with PBS. The data presented in the Table below are the average of two separate experiments and demonstrate that the antisense oligonucleotides do not target the WT rhodopsin gene.

Table 41
% inhibition of human WT rhodopsin expression

ISIS No	Chemistry	Dose (μg)	% inhibition
564426	3-10-3 cEt	50	13
		35	13
664844	Deoxy, MOE, and cEt	50	16
		35	17
664867	Deoxy, 2'-alpha-fluoro and cEt	50	12
		35	3
664884	Deoxy, MOE, and cEt	50	14
		35	1

Example 10: Tolerability study of antisense oligonucleotides targeting human mutant P23H rhodopsin in cynomolgus monkeys

Cynomolgus monkeys were treated with ISIS antisense oligonucleotides selected from studies described in the Examples above. The objective of this study was to determine the tolerability of the antisense oligonucleotides when given as a single intravitreal injection to cynomolgous monkeys. A cynomolgus surrogate ASO, ISIS 602881, was included in the study.

At the time this study was undertaken, the cynomolgus monkey genomic sequence was not available in the National Center for Biotechnology Information (NCBI) database; therefore, cross-reactivity with the cynomolgus monkey gene sequence could not be confirmed. Instead, the sequences of the ISIS antisense oligonucleotides used in the cynomolgus monkeys was compared to a rhesus monkey sequence for homology. It is expected that ISIS oligonucleotides with homology to the rhesus monkey sequence are fully cross-reactive with the cynomolgus monkey sequence as well. The human antisense oligonucleotides tested are cross-reactive with the rhesus genomic sequence (the complement of GENBANK Accession No. NW_001096632.1 truncated from nucleotides 1522000 to 1532000, designated herein as SEQ ID NO: 4). The greater the complementarity between the human oligonucleotide and the rhesus monkey sequence, the more likely the human oligonucleotide can cross-react with the cynomolgus monkey sequence. “Start site” indicates the 5’-most nucleotide to which the gapmer is targeted in the rhesus monkey gene sequence. ‘Mismatches’ indicates the number of nucleobases mismatched between the human oligonucleotide sequence and the rhesus monkey genomic sequence.

Table 42
Antisense oligonucleotides complementary to the rhesus rhodopsin genomic sequence (SEQ ID NO: 4)

ISIS No	Target Start Site	Mismatches	Sequence	Chemistry	SEQ ID NO
564426	1525	1	TACTCGAAGTGGCTGC	3-10-3 cEt	15
664867	1525	1	TACUCGAAGTGGCTGC	Deoxy, 2’-alpha-fluoro and cEt	64
664884	1525	1	ACTCGAAGTGGCTGC	Deoxy, MOE and cEt	29
664844	1527	1	GGTACTCGAAGTGGCT	Deoxy, MOE and cEt	21
602881	6434	0	TCATTCTGCACAGGCG	3-10-3 cEt	70

Treatment

Prior to the study, the monkeys were kept in quarantine during which the animals were observed daily for general health. The monkeys were 2-4 years old and weighed between 2 and 6 kg. The monkeys were randomized and assigned to groups, as shown the Table below. The monkeys were injected in the left eye (OS) with either PBS or various ASO doses and in the right eye (OD) with various ASO doses. ’OS’ stands for ‘oculus sinister’ (left eye) and ‘OD’ stands for ‘oculus dexter’ (right eye).

Table 43
Monkey groups

Group No.	ISIS No	Test material OS/OD	Dose OS/OD (μg/eye)	No. of animals
1	564426	PBS/ASO	0/150	4
2		ASO/ASO	450/450	3
3		ASO/ASO	750/750	2
4		ASO/ASO	1500/1500	1
5	664844	PBS/ASO	0/150	4
6		ASO/ASO	450/450	3
7		ASO/ASO	750/750	2
8		ASO/ASO	1500/1500	1
9	664867	PBS/ASO	0/150	4
10		ASO/ASO	450/450	3
11		ASO/ASO	750/750	2
12		ASO/ASO	1500/1500	1
13	664884	PBS/ASO	0/150	4
14		ASO/ASO	450/450	3
15		ASO/ASO	750/750	2
16		ASO/ASO	1500/1500	1
17	602881	PBS/ASO	0/400	4

Doses were administered on Day 1. Food was withheld prior to sedation. The animals were sedated with ketamine and dexdomitor for the dosing procedure. The eyes were cleansed with Betadine® and rinsed with sterile saline. Prior to the dose administration, a mydriatic (1% tropicamide) was instilled in each eye, followed by a topical anesthetic. An intravitreal injection of ASO or PBS was administered in each eye. A lid speculum was inserted to keep the lids open during the procedure and the globe was retracted. The needle was inserted through the sclera and pars plana approximately 4 mm posterior to the limbus. The needle was directed posterior to the lens into the mid vitreous. The test material was slowly injected into the mid-vitreous. Forceps were used to grasp the conjunctiva surrounding the syringe prior to needle withdrawal. Following dosing, all eyes were examined with an indirect ophthalmoscope to identify any visible post-dosing problems and confirm test material deposition. Sedation was reversed with antisedan. A topical antibiotic was dispensed onto each eye immediately following dosing and one day after dosing to prevent infection.

RNA Analysis

On day 70, eyes were collected within 10 min of exsanguination, rapidly frozen by submersion in liquid nitrogen, and placed on dry ice. Eyes were harvested from monkeys that had been treated with 150 μg or 450 µg of ISIS 564426, ISIS 664844, ISIS 664867, ISIS 664884 and 400 µg of ISIS 602881. RNA was extracted from the eye tissue for real-time PCR analysis of mRNA expression. The data from the PBS control eyes were evaluated and the average was calculated. Results are presented as percent inhibition of mRNA, relative to the PBS control, normalized to cone rod homeobox expression. A '0' value inhibition only indicates that the oligonucleotide did not inhibit expression of in this particular instance.

Table 44
% rhodopsin inhibition compared to PBS control

ISIS No	Dose (µg)	% inhibition
564426	150	0
	450	25
664844	150	8
	450	14
664867	450	21
664884	150	10
	450	46
602881	400	54

Electroretinography (ERG)

The potential effect of the antisense oligonucleotides on ocular tolerability was determined by measuring the ERG response of the animals following 9 weeks of treatment. The light-adapted b-wave ERG response provided an assessment of the function of the cone photoreceptors and the bipolar cells in the eye (Hood and Birch, Visual Neuroscience. 1992. 8: 107-126; Bouskila et al., Plos One 2014. 9: e111569). Electroretinograms (ERGs) were recorded using a UTAS E-3000 Visual Electrodiagnostic System. Light-adapted b-wave ERG responses in anesthetized monkeys were measured after stimulation with white light at luminance intensity of 2.7 cd.m^2.

The results are presented in the Table below as percent of baseline amplitude (means ± SD). As shown in the Table below, at the higher dosage of 750 µg of ISIS 564426, ISIS 664867 and ISIS 664884 per eye, the b-wave response trended towards lower levels. Furthermore, response in animals treated with ISIS 564426 trended lower at a dose of 450 µg per eye. These results indicate that ISIS 664844 is more tolerable than ISIS 564426, ISIS 664867, or ISIS 664884.

Table 45
Light-adapted (photopic) b-wave amplitude (% baseline)

ISIS No	Dose Level (µg/eye)			
	0	150	450	750
564426	88 ± 24	94 ± 27	50 ± 18	48 ± 19
664844	111 ± 43	87 ± 36	78 ± 13	106 ± 47
664867	83 ± 28	69 ± 14	53 ± 18	25 ± 26

664884	84 ± 7	107 ± 41	82 ± 29	35 ± 24

Pathology

After exsanguination, eyes with bulbar conjunctivae and attached optic nerve were collected from various groups and preserved in modified Davidson's fixative for 48-72 hours. The tissues were then transferred to 70% alcohol for at least 24 hours prior to processing to paraffin block. The paraffin-embedded samples were sectioned parallel to the ciliary artery to include optic nerve, macula, and optic disc. After the section was faced, 5 sections at approximately 30-micron steps, were collected. The sections were mounted on glass slides, stained with hematoxylin and eosin and analyzed for histopathology. The findings are presented in the Table below. 'OS' indicates 'outer stripe'; 'IS' indicates 'Inner stripe'; 'ONL' indicates 'outer nuclear layer'; 'INL' indicates 'inner nuclear layer'; 'GCL' indicates 'ganglion cell layer'. These results indicate that ISIS 664844 is more tolerable than ISIS 564426, ISIS 664867, or ISIS 664884.

Table 46
Pathology findings in monkey screening study

ISIS No	Dose/eye		
	450 µg	750 µg	1500 µg
564426	Not remarkable	Not remarkable	Min decreased cellularity ONL
664844	Not remarkable	Not remarkable	Not remarkable
664867	Not remarkable	Slightly decreased cellularity ONL	Slightly decreased cellularity ONL
664884	Not remarkable	Slightly decreased cellularity ONL; slight vacuolation ONL	Loss of ONL, IS and OS; Slight decreased cellularity GCL and INL

Additional tolerability assays

Ophthalmic examinations were conducted by an Ophthalmology Individual Scientist once during pretreatment, during week 1 (within 2-4 days following dose administration), and during weeks 3, 6, and 9. The animals were lightly sedated with ketamine prior to this procedure. Slit lamp biomicroscopy and indirect ophthalmoscopy was used. The anterior segment was scored using the Hackett McDonald scale (Hackett, R.B. and McDonald, T.O. 1996. "Assessing Ocular Irritation" in: Dermatotoxicology. 5th edition. Ed. By F. B. Marzuli and H.I. Maiback. Hemisphere Publishing Corp., Washington, D.C.).

Tonometry assessments were performed once pretreatment and during weeks 3 and 9 at approximately the same time of day. Intraocular pressure (IOP) measurements were performed on sedated animals using a pneumotonometer under laboratory light conditions.

Pachymetry (corneal thickness) measurements were performed once pretreatment and during weeks 6 and 9. Measurements of the central cornea was performed on sedated animals.

Non-contact Specular Microscopy (NCSM) was performed once pretreatment and during weeks 5 and 9.

All the assessments are tabulated below. A '√' sign indicates acceptable results; a 'X' indicates not acceptable. The results indicate that ISIS 664844 is more tolerable compared to ISIS 564426, ISIS 664867, or ISIS 664884.

Table 47
Tolerability screen in monkey study

Test	Utility	ISIS 564426	ISIS 664844	ISIS 664867	ISIS 664884	ISIS 602881
Ophthalmic Exam	Cataracts, major retina or vitreous abnormalities	√	√	√	X	√
Tonometry	IOP	√	√	√	√	√
Pachymetry	Corneal thickness	√	√	√	√	√
NCSM	Corneal endothelial cellularity, corneal thickness	√	√	√	√	X
Histology	Cellularity changes	√	√	X	X	√

Example 11: Screening summary

Over 400 antisense oligonucleotides (>200 ASOs having a MOE sugar modification and > 200 ASOs having a cEt modification) were screened as described in Examples 1-10 above. Out of more than 400 ASOs, ISIS 664844 exhibited the best combination of properties in terms of potency, tolerability, and selectivity for P23H rhodopsin.

WHAT IS CLAIMED:

1. A compound comprising (i) a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 15, 21, or 64, or (ii) a modified oligonucleotide consisting of 15 to 30 linked nucleosides having a nucleobase sequence comprising SEQ ID NO: 29,

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.

2. A compound comprising a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 15, 21, 29, or 64,

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.

3. The compound of any one of claims 1-2, wherein the oligonucleotide is at least 90% complementary to any one of SEQ ID NOs: 1-3.

4. The compound of any one of claims 1-3, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar, or at least one modified nucleobase.

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

6. The compound of claim 4 or 5, wherein the modified sugar is a bicyclic sugar.

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7. The compound of claim 6, wherein the bicyclic sugar is selected from the group consisting of: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)$_2$-O-2' (ENA); and 4'-CH(CH_3)-O-2' (cEt).

8. The compound of claim 4, wherein the modified sugar is 2'-O-methoxyethyl.

9. The compound of any one of claims 4-8, wherein the modified nucleobase is a 5-methylcytosine.

10. The compound of claim 1, wherein the modified oligonucleotide consists of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 15, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three 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 cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

11. The compound of claim 1, wherein the modified oligonucleotide consists of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 64, wherein the modified oligonucleotide comprises:

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt sugar, a cEt sugar, a cEt sugar, and a 2'-flouro sugar in the 5' to 3' direction; wherein each nucleoside of the 3' wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

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12. A compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 21, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, a cEt sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

13. The compound of claim 1, wherein the modified oligonucleotide consists of 15 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 29, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of the 5' wing segment comprises a cEt sugar; wherein the 3' wing segment comprises a cEt sugar, a 2'-O-methoxyethyl sugar, and a cEt sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

14. The compound of any one of claims 1-13, wherein the compound consists of the modified oligonucleotide.

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

16. Use of the compound of any one of claims 1-14 or the composition of claim 15 for the treatment, amelioration, or slowing progression of retinitis pigmentosa (RP) in a subject.

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17. The use of claim 16, wherein the retinitis pigmentosa is autosomal dominant retinitis pigmentosa (AdRP).

18. The use of claim 17, wherein the AdRP is associated with P23H rhodopsin.

19. The use of any one of claim 16-18, wherein the subject has a P23H rhodopsin allele.

20. The use of any one of claims 16-19, wherein the use of the compound or composition improves, or preserves worsening of visual function, visual field, photoreceptor cell function, electroretinogram (ERG) response, or visual acuity.

21. The use of any one of claims 16-20, wherein the use of the compound or composition inhibits, or delays progression of photoreceptor cell loss or deterioration of the retina outer nuclear layer.

22. The use of any one of claims 16-20, wherein the use of the compound or composition selectively inhibits expression of P23H rhodopsin over wild-type rhodopsin in the subject.

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