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(54) Titre : MODULATION DE L'EXPRESSION DE L'ALPHA SYNUCLEINE
(54) Title: MODULATION OF ALPHA SYNUCLEIN EXPRESSION

(57) Abrégé/Abstract:

Disclosed herein are antisense compounds and methods for decreasing alpha- synuclein mRNA and protein expression. Also disclosed herein are methods for treating, preventing, and ameliorating neurodegenerative diseases in an individual in need thereof.

ABSTRACT

Disclosed herein are antisense compounds and methods for decreasing alpha-synuclein mRNA and protein expression. Also disclosed herein are methods for treating, preventing, and ameliorating neurodegenerative diseases in an individual in need thereof.

MODULATION OF ALPHA SYNUCLEIN EXPRESSION

This application is a divisional of Canadian Patent Application No. 2,817,960, filed November 17, 2011.

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 BIOL0139WOSEQ.txt created 11/17/2011, which is 170 Kb in size and forms part of the description.

Field of the Invention

Embodiments of the present invention provide methods, compounds, and compositions for inhibiting expression of alpha-synuclein mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate neurodegenerative diseases, including, Parkinson's disease, dementia, multiple system atrophy, and Alzheimer's disease.

Background of the Invention

Alpha-synuclein (also known as α-synuclein, SNCA, and a-SYN) is a small, highly charged 140-amino acid residue protein, predominantly expressed in central nervous system (CNS) neurons, where it is localized at presynaptic terminals in close proximity to synaptic vesicles (Iwai, et al., *Neuron.* 1995. 14: 467-475). Alpha-synuclein can associate with lipid membranes by forming amphipathic α-helices, as shown in vitro (Davidson, et al., *J. Biol. Chem.* 1998. 273: 9443-9449). Although the function of alpha-synuclein is still poorly understood, several studies suggest that it is involved in modulating synaptic transmission, the density of synaptic vesicles, and neuronal plasticity (Cabin et al., *J. Neurosci.* 2002. 22: 8797-8807). It has also been suggested that alpha-synuclein may have a chaperone function, as indicated by its effectiveness in preventing aggregation of proteins in *in vitro* assays (Souza et al., *FEBS Lett.* 2000. 474: 116-119). Moreover, *in vivo* assays demonstrate that alpha-synuclein chaperone activity is instrumental in promoting the assembly of the SNARE-complex, which is essential for neurotransmitter release in the presynaptic terminals of the brain (Burre et al., *Science.* 329: 1663-1667). Decreased SNARE-complex assembly is associated with neurological impairment, thus, indicating a link between presynaptic alpha-synuclein aggregates and neurodegeneration (Kramer and Schulz-Schaeffer, *J.Neurosci.* 2007. 27: 1405-1410). Knockout mouse models of alpha-synuclein are not lethal, and brain morphology is intact, suggesting that alpha-synuclein is not required for neuronal development and/ or that compensatory pathways are present (Abeliovich et al., *Neuron.* 2000. 25: 239-252).

Misfolding, aggregation, and fibrillation of alpha-synuclein are implicated as critical factors in several neurodegenerative diseases, including, Parkinson's disease, Lewy body variant of Alzheimer's disease, diffuse Lewy body disease, dementia with Lewy bodies, and multiple system atrophy (Schulz-Schaeffer *Acta Neuropathol.* 2010. 120: 131-143; Yoshida. *Neuropathology.* 2007. 27: 484-493). In each of these cases, alpha-synuclein protein is misfolded and assembles in aggregates in Lewy bodies and Lewy neurites (Uversky. *J. Neurochem.* 2007. 103: 17-37). Several recent studies have shown that lipidic environments that promote alpha-synuclein folding also accelerate alpha-synuclein aggregation, suggesting that the lipid-associated conformation of alpha-synuclein may be relevant to alpha-synuclein misfolding in neurodegenerative diseases (Conway et al., *Science.* 2001. 294: 6-9; Lee et al., *J. Biol. Chem.* 2002. 277: 671-678). Mutations at position 53, where alanine is changed to threonine, and at position 30, where alanine is changed to proline, have been shown to cause alpha-synuclein to be in a random coil state, so that aggregation is more likely to occur (Clayton and George, J. *Neurosci.* 1999. 58: 120-129).

There is a currently a lack of acceptable options for treating such neurodegenerative disorders. It is therefore an object herein to provide compounds and methods for the treatment of such diseases and disorder.

Summary of the Invention

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

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

Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such diseases, disorders, and conditions are neurodegenerative diseases, disorders, and conditions. In certain embodiments, such neurodegenerative diseases, disorders, and conditions include Parkinson's Disease, dementia, multiple system atrophy (also Shy-Drager syndrome), sporadic and familial Alzheimer's Disease, Lewy body variant of Alzheimer's disease, diffuse Lewy body disease, dementia with Lewy bodies, and pure autonomic failure (also known as Bradbury-Eggleston syndrome). In certain embodiments, such diseases, disorders, and conditions are termed synucleinopathies. In certain embodiments, such synucleinopathies include Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and pure autonomic failure.

Such diseases, disorders, and conditions can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of a neurodegenerative disease, and, in particular, a synucleinopathy, include older age, exposure to neurotoxins, genetic predisposition, and trauma.

In certain embodiments, methods of treatment include administering an alpha-synuclein specific inhibitor to an individual in need thereof.

Detailed Description of the Invention

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

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

Definitions

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

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

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

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

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

"Active pharmaceutical agent" means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to an alpha-synuclein nucleic acid is an active pharmaceutical agent.

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

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

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

"Alpha-synuclein nucleic acid" or "α-synuclein" or "SNCA" or "a-SYN" means any nucleic acid encoding alpha-synuclein. For example, in certain embodiments, an alpha-synuclein nucleic acid includes a DNA sequence encoding alpha-synuclein, an RNA sequence transcribed from DNA encoding alpha-synuclein (including genomic DNA comprising introns and exons), and an mRNA sequence encoding alpha-synuclein. "alpha-synuclein mRNA" means an mRNA encoding an alpha-synuclein protein.

"Alpha-synuclein specific inhibitor" refers to any agent capable of inhibiting the expression of alpha-synuclein mRNA and/or alpha-synuclein protein with few to no off-target effects. Alpha-synuclein specific inhibitors include, but are not limited to, nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of alpha-synuclein mRNA and/or alpha-synuclein protein. In certain embodiments, by specifically modulating alpha-synuclein mRNA expression and/or alpha-synuclein protein expression, alpha-synuclein specific inhibitors affect other downstream proteins and molecules.

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

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

"Antibody" refers to a molecule characterized by reacting specifically with an antigen in some way, where the antibody and the antigen are each defined in terms of the other. Antibody may refer to a complete antibody molecule or any fragment or region thereof, such as the heavy chain, the light chain, Fab region, and Fc region.

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

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

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

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

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

"Bicyclic nucleoside" means a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring.

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

"cEt" or "constrained ethyl" means a bicyclic nucleoside having a 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'.

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

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

"Co-administration" means administration of two or more pharmaceutical agents to an individual. The two or more pharmaceutical agents may be in a single pharmaceutical composition, or may be in separate pharmaceutical compositions. Each of the two or more pharmaceutical agents may be administered through the same or different routes of administration. Co-administration encompasses parallel or sequential administration.

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

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

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

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

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

"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."

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

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

"Identifying an animal at risk for neurodegenerative disease" means identifying an animal having been diagnosed with a neurodegenerative disease or identifying an animal predisposed to develop a neurodegenerative disease. 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.

"Inhibiting alpha-synuclein" means reducing expression of alpha-synuclein mRNA and/or protein levels in the presence of an alpha-synuclein specific inhibitor as compared to expression of alpha-synuclein mRNA and/or protein levels in the absence of an alpha-synuclein specific inhibitor.

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

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

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

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

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

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

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

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

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

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

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

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

"Neurodegenerative disease" means a disease characterized by progressive loss of structure or function of neurons, including death of neurons.

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

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

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

"Nucleoside" means a nucleobase linked to a sugar.

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

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

"Off-target effect" refers to an unwanted or deleterious biological effect associated with modulation of RNA or protein expression of a gene other than the intended target nucleic acid.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Certain Embodiments

Embodiments of the present invention provide methods, compounds, and compositions for inhibiting alpha-synuclein mRNA and protein expression.

Embodiments of the present invention provide methods for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with alpha-synuclein in an individual in need thereof. Also contemplated are methods for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with alpha-synuclein. Alpha-synuclein associated diseases, disorders, and conditions include neurodegenerative diseases and synucleinopathies, which include Parkinson's Disease, dementia, multiple system atrophy (also Shy-Drager syndrome), sporadic and familial Alzheimer's Disease, Lewy body variant of Alzheimer's disease, diffuse Lewy body disease, and dementia with Lewy bodies.

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

In certain embodiments of the present invention, alpha-synuclein specific inhibitors are peptides or proteins, such as, but not limited to, synthetic construct alpha-synuclein (68-78), N-methylated at G1y73 as described in *Neurosci. Lett.* 2004. 359: 89-93; N-methylated derivative of SNCA (25-35) as described in *J. Biol. Chem.* 2000. 275: 25109-25112; ASI peptides as described in *FASEB J.* 2004. 18: 1315-1317; RGAVVTGR-amide and RGGAVVTGRRRRRR-amide as described in *Biochem. Soc. Trans.* 2005. 33: 1106-1110; FK506 as described in *J. Neurosci.* 2010. 30: 2454-2463; tissue transglutaminase as described in *Protein Sci.* 2008. 17: 1395-1402; beta-synuclein as described in *J. Biol. Chem.* 2005. 280: 7562-7569; and peptidyl compounds which are retroenantiomers of the alpha-synuclein sequence as described in US 2009/0286745.

In certain embodiments of the present invention, alpha-synuclein specific inhibitors are antibodies, such as, but not limited to, human single-chain Fv (scFv) antibody, D10, as described in *Mol. Ther.* 2004. 10: 1023-1031; human alpha-SNCA antibodies as described in USPN 7,727,957; anti-synuclein antibodies as described in USPN 6,890,535; humanized or chimeric 9E4 antibody as described in USPPN 2010/0278814; humanized version of mouse monoclonal antibody 6H7 as described in USPPN 2010/0031377; and humanized anti-synuclein monoclonal antibody as described in USPPN 2008/0300204.

In certain embodiments of the present invention, alpha-synuclein specific inhibitors are small molecules, such as, but not limited to, curcumin, nicotine, and wine-related polyphenols as described in *Curr. Pharm. Des.* 2008. 14: 3247-3266; 4% H_2O_2 as described in *Biochim. Biophys. Acta* 2005. 1703: 157-169; selegiline as described in *J. Mol. Biol.* 2010. Nov. 1st. Epub ahead of print; baicalein as described in *J. Neurochem.* 2010. 114: 419-429; cyclic tetrapyrrole phthalocyanine tetrasulfonate as described in *Proc. Natl. Acad. Sci* USA. 2009. 106: 1057-62; SNX-0723 as described in *J. Pharmacol. Exp. Ther.* 2010. 332: 849-857; N'-benzylidene-benzohydrazide compounds as described in *Biochem. Biophys. Res. Commun.* 2010. 391: 461-466; MG132 and epoxomicin as described in *Neurotox. Res.* 2010. 17: 215-227; congo red and Lacmoid as described in *Biochemistry.* 2009. 48: 8322-8334; flavonoid quinine as described in *Biochemistry.* 2009. 48: 8206-8224; valproic acid as described in *Neurotox. Res.* 2010. 17: 130-141; 3,4-dihydroxyphenylacetic acid (DOPAC) as described in *J. Mol. Biol.* 2009. 388: 597-610; PAMAM dendrimers as described in *Macromol. Biosci.* 2009. 9: 230-238; dopamine as described in *PLoS One.* 2008. 3: e3394; melatonin as described in *J. Pineal Res.* 2007. 42: 125-130; rifampicin as described in *Brain Res.* 2007. 1139: 220-225 and *Chem. Biol.* 2004. 11: 1513-1521; ganglioside GM1 as described in *Biochemistry.* 2007. 46: 4868-1877; 4-hydroxy-2-nonenal as described in *J. Biol. Chem.* 2007. 282: 5862-5870; trehalose as described in *J. Biol. Chem.* 2007. 282: 5641-5652; 1,2-dipalmitoyl-sn-glycero-3-phosphate/1,2-dipalmitoyl-sn-glycero-3-phosphocholine and 1,2-dipalmitoyl-sn-glycero-3-phospho-RAC-(1-glycerol)/1,2-dipalmitoyl-sn-glycero-3-phosphocholine as described in *J. Biol. Chem.* 2003. 278: 16873-16877; bis- and tris-dihydroxyaryl compounds and their methylenedioxy analogs as described in USPPN 2010/0179223 and USPN 7,763,747; 5-(fluoromethyl)piperidine-3,4-diol, 5-(chloromethyl)piperidine-3,4-diol as described in USPPN 2010/0261753; ramelteon as described in USPPN 2010/0056622; cleavage agents as described in USPPN 2010/0036122; Uncaria tomentosa extract, gingko biloba, green tea extract, grape seed extract and curcumin as described in USPPN 2009/0123575; catechin or green tea extract as described in USPPN 2008/0306143; farncsyl transferase inhibitor as described in USPPN 2007/0213366.

Embodiments of the present invention provide antisense compounds targeted to an alpha-synuclein nucleic acid. In certain embodiments, the alpha-synuclein nucleic acid is any of the sequences set forth in GENBANK Accession No. NM_000345.3, incorporated herein as SEQ ID NO: 1; the complement of GENBANK Accession No. NT_016354.17 truncated from nucleotides 15140000 to 15255000, incorporated herein as SEQ ID NO: 2; GENBANK Accession No. NM_007308.1, incorporated herein as SEQ ID NO: 3; GENBANK Accession No. L36674.1, incorporated herein as SEQ ID NO: 4; GENBANK Accession No. BC013293.2, incorporated herein as SEQ ID NO: 5; GENBANK Accession No. BG701026.1, incorporated herein as SEQ ID NO: 6; or GENBANK Accession No. BM069769.1, incorporated herein as SEQ ID NO: 7.

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

In certain embodiments, the compound of the invention may comprise a modified oligonucleotide comprising a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% complementary to an equal length portion of SEQ ID NO: 1, 2, 3, 4, 5, 6, or 7. In certain embodiments, the compound of the invention may comprise a modified oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1, 2, 3, 4, 5, 6, or 7.

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 404 to 463 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 404 to 463 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 40% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in SH-SY5Y cells (e.g., as described in Example 6).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 107 to 126 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 107 to 126 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 236 to 301 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 236 to 301 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 304 to 331 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 304 to 331 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ

ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 361 to 400 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 361 to 400 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 70% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 404 to 423 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 404 to 423 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 90% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 444 to 463 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least

20 contiguous nucleobases complementary to an equal length portion of nucleobases 444 to 463 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 90% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 469 to 488 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 469 to 488 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 90% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 542 to 573 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 542 to 573 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 60% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 607 to 721 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 607 to 721 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 30% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 734 to 837 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 734 to 837 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 30% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 881 to 927 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 881 to 927 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ

ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 60% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 952 to 983 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 952 to 983 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 40% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1001 to 1020 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1001 to 1020 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1030 to 1049 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least

20 contiguous nucleobases complementary to an equal length portion of nucleobases 1030 to 1049 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 30% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1055 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1055 to 1091 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 80% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1242 to 1261 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1242 to 1261 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 20% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1292 to 1333 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1292 to 1333 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 20% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1345 to 1374 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1345 to 1374 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 20% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1432 to 1501 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1432 to 1501 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of

SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 30% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1522 to 1541 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1522 to 1541 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 40% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

In certain embodiments, the invention provides a compound comprising a modified oligonucleotide comprising a nucleobase sequence complementary to at least a portion of nuncleobases 1703 to 1742 of SEQ ID NO: 1. Said modified oligonucleotide may comprise at least 8, at least 10, at least 12, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1703 to 1742 of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may comprise a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NO: 1. Said modified oligonucleotide may achieve at least 60% inhibition of human mRNA levels as determined using an RT-PCR assay method, optionally in HuVEC cells (e.g., as described in Example 1).

Embodiments of the present invention provide, a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 11 to 88 and 98 to 136.

In certain embodiments, the modified oligonucleotide is a single-stranded oligonucleotide.

In certain embodiments, the modified oligonucleotide has a nucleobase sequence that is 100% complementary to a human alpha-synuclein nucleic acid.

In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage.

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

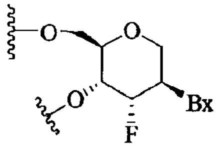
In certain embodiments, the at least one nucleoside of the modified oligonucleotide comprises a modified sugar.

In certain embodiments, the modified sugar is a bicyclic sugar.

In certain embodiments, the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' bridge.

In certain embodiments, the at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

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



wherein Bx is an optionally protected heterocyclic base moiety.

In certain embodiments, the modified sugar comprises a 2'-O-methoxyethyl group.

In certain embodiments, the at least one nucleoside of the modified oligonucleotide comprises a modified nucleobase.

In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide comprises:

(i) a gap segment consisting of linked deoxy nucleosides;

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

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

In certain embodiments, the modified oligonucleotide comprises:

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

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

Embodiments of the present invention provide methods for identifying an animal having a neurodegenerative disease and administering to said animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: SEQ ID NOs: 11 to 88 and 98 to 136.

In certain embodiments, the administration reduces expression of alpha-synuclein.

In certain embodiments, the administration improves motor coordination.

In certain embodiments, the administration improves olfaction.

In certain embodiments, the administration improves spatial memory.

In certain embodiments, the administration reduces aggregation of alpha-synuclein.

Embodiments of the present invention provide, a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising a portion of at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases complementary to an equal length portion of nucleobases 404 to 463 of SEQ ID NO: 1; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to SEQ ID NO: 1.

Embodiments of the present invention provide, the use of any antisense oligonucleotide described herein for reducing expression of alpha-synuclein in an animal.

Embodiments of the present invention provide, the use of any antisense oligonucleotide described herein for improving motor coordination in an animal.

Embodiments of the present invention provide, the use of any antisense oligonucleotide described herein for reducing aggregation of alpha-synuclein in an animal.

Embodiments of the present invention provide, the use of any antisense oligonucleotide described herein for use in treating an animal having a disease or condition associated with alpha-synuclein by administering to the animal a therapeutically effective amount of the compound so that expression of alpha-synuclein is inhibited.

Antisense Compounds

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

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

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

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

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

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

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

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

Antisense Compound Motifs

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

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

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

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

In certain embodiments, antisense compounds targeted to an alpha-synuclein nucleic acid possess a 5-10-5 gapmer motif.

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

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode alpha-synuclein include, without limitation, the following: GENBANK Accession No. NM_000345.3, incorporated herein as SEQ ID NO: 1; the complement of GENBANK Accession No. NT_016354.17 truncated from nucleotides 15140000 to 15255000, incorporated herein as SEQ ID NO: 2; GENBANK Accession No. NM_007308.1, incorporated herein as SEQ ID NO: 3; GENBANK Accession No. L36674.1, incorporated herein as SEQ ID NO: 4; GENBANK Accession No. BC013293.2, incorporated herein as SEQ ID NO: 5; GENBANK Accession No. BG701026.1, incorporated herein as SEQ ID NO: 6; or GENBANK Accession No. BM069769.1, incorporated herein as SEQ ID NO: 7.

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

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

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

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

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

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

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in alpha-synuclein mRNA levels are indicative of inhibition of alpha-synuclein expression. Reductions in levels of an alpha-synuclein protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of alpha-synuclein expression. For example, improved motor coordination, reduced incidence of resting tremor, reduced incidence of bradykinesia (slow movement), reduced rigidity or inflexibility, improved balance, improved fine motor dexterity, improved gross motor coordination, reduced aggregation of alpha-synuclein, recovery from loss in olfaction, and improved autonomic function, such as, decreased orthostatic hypotension.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and an alpha-synuclein 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 an alpha-synuclein 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 an alpha-synuclein nucleic acid).

Non-complementary nucleobases between an antisense compound and an alpha-synuclein 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 an alpha-synuclein 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 an alpha-synuclein 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 an alpha-synuclein nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

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

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

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

Identity

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

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

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

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

Modifications

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

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

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

Modified Internucleoside Linkages

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

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

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

Modified Sugar Moieties

Antisense compounds of the invention can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring moiety. Examples of chemically modified ribofuranose rings include, without limitation, addition of substitutent groups (including 5' and 2' substituent groups); bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA); replacement of the ribosyl ring oxygen atom with S, N(R), or C(R1)(R)2 (R = H, 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), replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (*see*, published U.S. Patent Application US2005/0130923, published on June 16, 2005), or, alternatively, 5'-substitution of a BNA (*see*, PCT International Application WO 2007/134181, published on 11/22/07, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include, without limitation, nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3, 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, $O(CH_2)2SCH_3$, $O(CH_2)2$-O-N(Rm)(Rn), and O-CH_2-C(=O)-N(Rm)(Rn), where each Rm and Rn 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 wherein the bridge comprises a 4' to 2' bicyclic nucleoside. Examples of such 4' to 2' bicyclic nucleosides, include, but are not limited to, one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' and 4'-$CH(CH_2OCH_3)$-O-2', and analogs thereof (*see*, U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2', and analogs thereof (*see*, published PCT International Application WO2009/006478, published January 8, 2009); 4'-CH_2-$N(OCH_3)$-2', and analogs thereof (*see*, published PCT International Application WO2008/150729, 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 PCT International Application WO 2008/154401, published on December 8, 2008). Also 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., 129(26)* 8362-8379 (Jul. 4, 2007); Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; U.S. Patent Nos U.S. 6,670,461, 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 7,399,845; published PCT International applications WO 2004/106356, WO 94/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication

Nos. US2004/0171570, US2007/0287831, and US2008/0039618; and U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Application Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922. 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 $-[C(R_a)(R_b)]_n-$, $-C(R_a)=C(R_b)-$, $-C(R_a)=N-$, $-C(=NR_a)-$, $-C(=O)-$, $-C(=S)-$, $-O-$, $-Si(R_a)_2-$, $-S(=O)_x-$, and $-N(R_a)-$;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

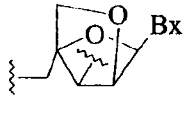
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle 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, (F) Methyl(methyleneoxy) (4'-CH(CH_3)-O-2') BNA, (G) methylene-thio (4'-CH_2-S-2') BNA, (H) methylene-amino (4'-CH2-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH_2-CH(CH_3)-2') BNA, and (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA as depicted below.



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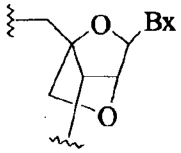

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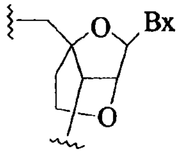

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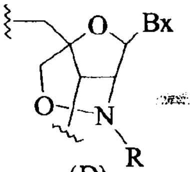

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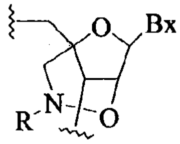

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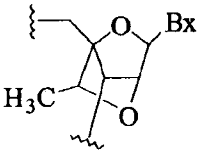

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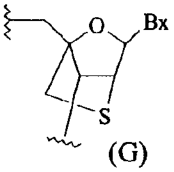

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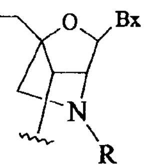
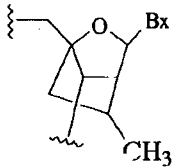
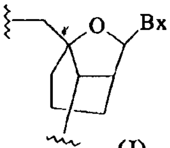
(H)





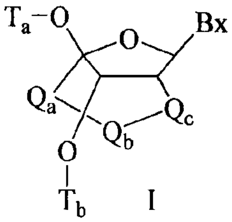
(I)



(J)

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

In certain embodiments, bicyclic nucleoside having Formula I:



I

wherein:

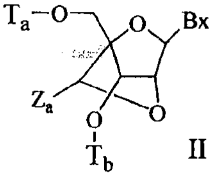
Bx is a heterocyclic base moiety;

$-Q_a-Q_b-Q_c-$ 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 nucleoside having Formula II:



II

wherein:

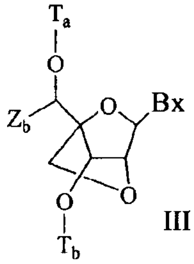
Bx is a heterocyclic base moiety;

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 nucleoside having Formula III:



III

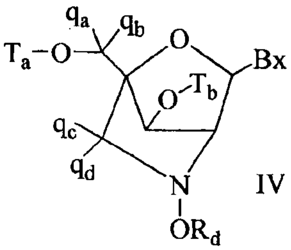
wherein:

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 nucleoside having Formula IV:



IV

wherein:

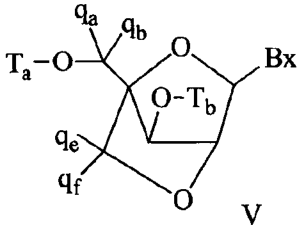
Bx is a heterocyclic base moiety;

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

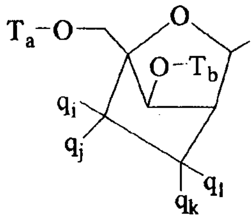
or q_e and q_f together are $=C(q_g)(q_h)$;

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 (*see, e.g.*, 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, methyleneoxy (4'-CH_2-O-2') BNA, and 2'-thio-BNAs, have also been prepared (*see, e.g.*, 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 (*see, e.g.*, 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 (*see, e.g.*, 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 nucleoside 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 (*see, e.g.*, 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 (*see, e.g.*, Srivastava *et al., J. Am. Chem. Soc.* 2007, *129(26)*, 8362-8379).

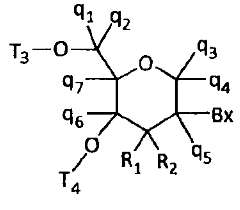
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting the 2' carbon atom and the 4' carbon atom.

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: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, 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; 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; and 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 (*see, e.g.*, 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 (*see, e.g.*, Martin, P., *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, CJ. *Bioorg. & Med. Chem.* (2002) **10**:841-854), fluoro HNA (F-HNA), or those compounds having Formula X:

Formula X:



X

wherein independently for each of said at least one tetrahydropyran 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 tetrahydropyran nucleoside analog to the antisense compound or one of T_3 and T_4 is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the 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;

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

one of R_1 and R_2 is hydrogen and the other is selected from 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 X are provided wherein q_m, q_n, q_p, q_r, q_s, q_t, and q_u are each H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t, and q_u is other than H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t and q_u is methyl. In certain embodiments, THP nucleosides of Formula X are provided wherein one of R_1 and R_2 is F. 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.

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.

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

As used herein, “MOE” or “2’-MOE” or “2’-$OCH_2CH_2OCH_3$” or “2’-O-methoxyethyl” each refers to a nucleoside comprising a sugar comprising a -$OCH_2CH_2OCH_3$ group at the 2’ position of the sugar ring.

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

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

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

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

In certain embodiments, antisense compounds comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a cEt. In certain embodiments, the cEt modified nucleotides are arranged throughout the wings of a gapmer motif.

Compositions and Methods for Formulating Pharmaceutical Compositions

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

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

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

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

Conjugated Antisense Compounds

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

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

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of alpha-synuclein nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commerical vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HuVEC cells and SH-SY5Y cells.

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.

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.

Analysis of inhibition of target levels or expression

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

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

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

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

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

Analysis of Protein Levels

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

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of alpha-synuclein and produce phenotypic changes, such as, improved motor coordination, improved olfaction, improved spatial memory, reduced incidence of resting tremor, reduced incidence of bradykinesia (slow movement), reduced rigidity or inflexibility, improved balance, improved fine motor dexterity, improved gross motor coordination, reduced aggregation of alpha-synuclein, and improved autonomic function, such as, decreased orthostatic hypotension. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, subcutaneous, intramuscular, intraarterial, or intracranial administration, e.g., intrathecal or intracerebroventricular administration. Calculation of antisense oligonucleotide dosage and dosing frequency depends upon many factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from liver tissue and changes in alpha-synuclein nucleic acid expression are measured. Changes in alpha-synuclein protein levels are also measured.

Certain Indications

In certain embodiments, the invention provides methods, compounds, and compositions of treating an individual comprising administering one or more pharmaceutical compositions of the present invention. In certain embodiments, the individual has a neurodegenerative disease. In certain embodiments, the neurodegenerative disease is Parkinson's Disease, dementia, multiple system atrophy (also Shy-Drager syndrome), sporadic and familial Alzheimer's Disease, Lewy body variant of Alzheimer's disease, diffuse Lewy body disease, or dementia with Lewy bodies. In certain embodiments, the individual has a synucleinopathy. In certain embodiments, the synucleinopathy is Parkinson's Disease, dementia with Lewy bodies, or multiple system atrophy. In certain embodiments, the individual is at risk for developing a neurodegenerative disease and/or a synucleinopathy. This includes individuals having one or more risk factors for developing a neurodegenerative disease and/or synucleinopathy, including, include older age, exposure to neurotoxins, and genetic predisposition. In certain embodiments, the individual has been identified as in need of treatment for a neurodegenerative disease and/or synucleinopathy. In certain embodiments the invention provides methods for prophylactically reducing alpha-synuclein expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to an alpha-synuclein nucleic acid.

In certain embodiments, administration of an antisense compound targeted to an alpha-synuclein nucleic acid results in reduction of alpha-synuclein expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to an alpha-synuclein nucleic acid results in improved motor coordination, improved olfaction, improved spatial memory, reduced incidence of resting tremor, reduced incidence of bradykinesia (slow movement), reduced rigidity or inflexibility, improved balance, improved fine motor dexterity, improved gross motor coordination, reduced aggregation of alpha-synuclein, and improved autonomic function, such as, decreased orthostatic hypotension. In certain embodiments, administration of an alpha-synuclein antisense compound improves motor coordination, reduces incidence of resting tremor, reduces incidence of bradykinesia (slow movement), reduces rigidity or inflexibility, improves balance, improves fine motor dexterity, improves gross motor coordination, reduces aggregation of alpha-synuclein, improves autonomic function, and decreases orthostatic hypotension by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to alpha-synuclein are used for the preparation of a medicament for treating a patient suffering or susceptible to a neurodegenerative disease and/or synucleinopathy.

Administration

In certain embodiments, the compounds and compositions as described herein are administered parenterally.

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

In certain embodiments, compounds and compositions are delivered to the CNS. In certain embodiments, compounds and compositions are delivered to the cerebrospinal fluid. In certain embodiments, compounds and compositions are administered to the brain parenchyma. In certain embodiments, compounds and compositions are delivered to an animal by intrathecal administration, or intracerebroventricular administration. Broad distribution of compounds and compositions, described herein, within the central nervous system may be achieved with intraparenchymal administration, intrathecal administration, or intracerebroventricular administration.

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

Certain Combination Therapies

In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with one or more other pharmaceutical agents. In certain embodiments, one or more pharmaceutical compositions of the present invention are antisense oligonucleotides. In certain embodiments, one or more other pharmaceutical agents are any of peptides, antibodies, or small molecules. In certain embodiments, the peptides, antibodies, or small molecules are any of those described hereinabove (e.g., see *Certain Embodiments* above).

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

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

In certain embodiments, one more pharmaceutical compositions of the present invention are administered with physical therapy.

EXAMPLES

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.

Example 1: Antisense inhibition of human alpha-synuclein (SNCA) in HuVEC cells

Antisense oligonucleotides targeted to an SNCA nucleic acid were tested for their effects on SNCA mRNA *in vitro*. Cultured HuVEC cells at a density of 5,000 cells per well were transfected using LipofectAMINE2000® reagent with 10 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and SNCA mRNA levels were measured by quantitative real time PCR using the human primer probe set RTS2621 (forward sequence ACGAACCTGAAGCCTAAGAAATATCT, designated herein as SEQ ID NO: 8; reverse sequence GAGCACTTGTACAGGATGGAACAT, designated herein as SEQ ID NO: 9, probe sequence TGCTCCCAGTTTCTTGAGATCTGCTGACA, designated herein as SEQ ID NO: 10). SNCA mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of SNCA, relative to untreated control cells.

The chimeric antisense oligonucleotides in Tables 1, 2, and 3 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "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 in the human gene sequence. Each gapmer listed in Table 1 is targeted to SEQ ID NO: 1 (GENBANK Accession No. NM_000345.3). Each gapmer listed in Table 2 is targeted to SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_016354.17 truncated from nucleotides 15140000 to 15255000). Each gapmer listed in Table 3 is targeted to either SEQ ID NO: 3 (GENBANK Accession No. NM_007308.1), SEQ ID NO: 4 (GENBANK Accession No. L36674.1), SEQ ID NO: 5 (GENBANK Accession No. BC013293.2), SEQ ID NO: 6 (GENBANK Accession No. BG701026.1), or SEQ ID NO: 7 (GENBANK Accession No. BM069769.1).

As shown in Tables 1 and 2, several of the gapmers exhibited at least 50% inhibition, as measured by primer probe set RTS2621, including ISIS numbers: 387973, 387974, 387975, 387976, 387977, 387978, 387979, 387980, 387981, 387982, 387983, 387984, 387985, 387986, 387987, 387988, 387989, 387990, 387991, 387994, 387995, 387996, 387997, 387998, 387999, 388000, 388001, 388002, 388004, 388005, 388006, 388007, 388008, 388009, 388010, 388012, 388013, 388014, 388016, 388017, 388021, 388025, 388026, 388027, 388029, 388032, 388033, and 3880309.

Several of the gapmers exhibited at least 60% inhibition, including ISIS numbers: 387973, 387974, 387975, 387976, 387977, 387978, 387979, 387980, 387981, 387982, 387983, 387984, 387985, 387986, 387988, 387989, 387990, 387994, 387995, 387996, 387997, 387998, 387999, 388000, 388001, 388002, 388004, 388005, 388006, 388007, 388008, 388009, 388010, 388014, 388016, 388017, 388026, 388027, 388029, 388032, 388033, and 388039.

Several of the gapmers exhibited at least 70% inhibition, including ISIS numbers: 387973, 387974, 387975, 387976, 387977, 387978, 387979, 387980, 387981, 387982, 387983, 387984, 387985, 387986, 387989, 387994, 387995, 387996, 387997, 387998, 387999, 388000, 388001, 388004, 388006, 388008, 388009, 388010, 388014, 388016, 388017, 388027, 388029, and 388039.

Several of the gapmers exhibited at least 80% inhibition, including ISIS numbers: 387973, 387974, 387975, 387976, 387978, 387979, 387981, 387983, 387984, 387985, 387986, 387994, 387998, 387999, 388000, 388001, 388004, 388006, 388008, 388009, 388010, 388014, 388016, and 388017.

Several of the gapmers exhibited at least 90% inhibition, including ISIS numbers: 387973, 387975, 387983, 387984, 387985, 387986, 387994, 387998, and 388004.

Table 1

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

Start Site	Stop Site	Oligo ID	Sequence	% inhibition	SEQ ID NO
236	255	387973	AATTCCTTTACACCACACTG	92	11
246	265	387974	ATGGCTAATGAATTCCTTTA	89	12
256	275	387975	GAATACATCCATGGCTAATG	90	13
266	285	387976	GTCCTTTCATGAATACATCC	89	14
273	292	387977	TTTGAAAGTCCTTTCATGAA	78	15
282	301	387978	TCCTTGGCCTTTGAAAGTCC	88	16

304	323	387979	CTCAGCAGCAGCCACAACTC	80	17
312	331	387980	TTGGTTTTCTCAGCAGCAGC	77	18
361	380	387981	ATAGAGAACACCCTCTTTTG	83	19
375	394	387982	GTTTTGGAGCCTACATAGAG	77	20
381	400	387983	TCCTTGGTTTTGGAGCCTAC	91	21
404	423	387984	TTGCCACACCATGCACCACT	92	22
444	463	387985	CCAACATTTGTCACTTGCTC	95	23
469	488	387986	TGTCACACCCGTCACCACTG	96	24
542	561	387987	ACTGGTCCTTTTTGACAAAG	58	25
554	573	387988	CATTCTTGCCCAACTGGTCC	65	26
607	626	387989	GTCAGGATCCACAGGCATAT	78	27
622	641	387990	TTCATAAGCCTCATTGTCAG	63	28
629	648	387991	AAGGCATTTCATAAGCCTCA	52	29
637	656	387992	TTCCTCAGAAGGCATTTCAT	39	30
644	663	387993	GATACCCTTCCTCAGAAGGC	40	31
653	672	387994	CGTAGTCTTGATACCCTTCC	93	32
671	690	387995	TTTCTTAGGCTTCAGGTTCG	77	33
676	695	387996	AGATATTTCTTAGGCTTCAG	71	34
683	702	387997	GGAGCAAAGATATTTCTTAG	77	35
702	721	387998	AGCAGATCTCAAGAAACTGG	92	36
734	753	387999	ACTGAGCACTTGTACAGGAT	86	37
739	758	388000	TTGGAACTGAGCACTTGTAC	87	38
745	764	388001	GGCACATTGGAACTGAGCAC	87	39
764	783	388002	TTGAGAAATGTCATGACTGG	67	40
774	793	388003	TGTAAAAACTTTGAGAAATG	31	41
792	811	388004	GAAGACTTCGAGATACACTG	94	42
808	827	388005	TCAATCACTGCTGATGGAAG	66	43
818	837	388006	TACAGATACTTCAATCACTG	82	44
881	900	388007	GACCCTGCTACCATGTATTC	68	45
891	910	388008	AGCACACAAAGACCCTGCTA	88	46
897	916	388009	ATCCACAGCACACAAAGACC	80	47
908	927	388010	GAAGCCACAAAATCCACAGC	86	48
952	971	388011	GGTAGTCACTTAGGTGTTTT	49	49
958	977	388012	ATAAGTGGTAGTCACTTAGG	57	50
964	983	388013	TTAGAAATAAGTGGTAGTCA	57	51
1001	1020	388014	AACTTCTGAACAACAGCAAC	82	52
1030	1049	388015	CTTATAATATATGATAGCAA	34	53
1055	1074	388016	GTATCATTAAAAGACACCTA	86	54
1072	1091	388017	GTCATTATTCTTAGACAGTA	82	55
1242	1261	388018	TATTTTTGCAATGAGATAAC	28	56
1249	1268	388019	AATAAAATATTTTTGCAATG	0	57
1292	1311	388020	GCTTATAAGCATGATTTTTA	31	58

1302	1321	388021	AATTCATGTTGCTTATAAGC	51	59
1314	1333	388022	GTGTCAGTTCTTAATTCATG	20	60
1345	1364	388023	GGCTATTAATAACTTTATAT	29	61
1355	1374	388024	TTCTTCAAATGGCTATTAAT	45	62
1432	1451	388025	TTCTGGCAGTGTTGCTTCAG	59	63
1452	1471	388026	CAGTGCATACCAAAACACAC	61	64
1462	1481	388027	CTTAAGGAACCAGTGCATAC	77	65
1472	1491	388028	ATCACAGCCACTTAAGGAAC	31	66
1482	1501	388029	TCAATAATTAATCACAGCCA	70	67
1522	1541	388030	CCACTCTACAATAGTAGTTG	44	68
1693	1712	388031	TATCAGACAAAATAGATTTT	0	69
1703	1722	388032	TTCACACCAATATCAGACAA	67	70
1723	1742	388033	ATTGTCAGAAAGGTACAGCA	64	71
1733	1752	388034	AATATTATTTATTGTCAGAA	0	72
1741	1760	388035	CATGGTCGAATATTATTTAT	5	73
1170	1189	388037	TCGCAAAATGGTAAAATTTC	35	74
107	126	388039	GTCTGCGCTGCAGCCCGCAC	79	75

Table 2

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

Start Site	Stop Site	Oligo ID	Sequence	% inhibition	SEQ ID NO
3451	3470	387973	AATTCCTTTACACCACACTG	92	11
3461	3480	387974	ATGGCTAATGAATTCCTTTA	89	12
3471	3490	387975	GAATACATCCATGGCTAATG	90	13
3481	3500	387976	GTCCTTTCATGAATACATCC	89	14
3488	3507	387977	TTTGAAAGTCCTTTCATGAA	78	15
3497	3516	387978	TCCTTGGCCTTTGAAAGTCC	88	16
3519	3538	387979	CTCAGCAGCAGCCACAACTC	80	17
3527	3546	387980	TTGGTTTTCTCAGCAGCAGC	77	18
3576	3595	387981	ATAGAGAACACCCTCTTTTG	83	19
10958	10977	387983	TCCTTGGTTTTGGAGCCTAC	91	21
10981	11000	387984	TTGCCACACCATGCACCACT	92	22
16775	16794	387985	CCAACATTTGTCACTTGCTC	95	23
16800	16819	387986	TGTCACACCCGTCACCACTG	96	24
16873	16892	387987	ACTGGTCCTTTTTGACAAAG	58	25
109906	109925	387989	GTCAGGATCCACAGGCATAT	78	27
109921	109940	387990	TTCATAAGCCTCATTGTCAG	63	28
109928	109947	387991	AAGGCATTTCATAAGCCTCA	52	29
112485	112504	387994	CGTAGTCTTGATACCCTTCC	93	32
112503	112522	387995	TTTCTTAGGCTTCAGGTTCG	77	33

112508	112527	387996	AGATATTTCTTAGGCTTCAG	71	34
112515	112534	387997	GGAGCAAAGATATTTCTTAG	77	35
112534	112553	387998	AGCAGATCTCAAGAAACTGG	92	36
112566	112585	387999	ACTGAGCACTTGTACAGGAT	86	37
112571	112590	388000	TTGGAACTGAGCACTTGTAC	87	38
112577	112596	388001	GGCACATTGGAACTGAGCAC	87	39
112596	112615	388002	TTGAGAAATGTCATGACTGG	67	40
112606	112625	388003	TGTAAAAACTTTGAGAAATG	31	41
112624	112643	388004	GAAGACTTCGAGATACACTG	94	42
112640	112659	388005	TCAATCACTGCTGATGGAAG	66	43
112650	112669	388006	TACAGATACTTCAATCACTG	82	44
112713	112732	388007	GACCCTGCTACCATGTATTC	68	45
112723	112742	388008	AGCACACAAAGACCCTGCTA	88	46
112729	112748	388009	ATCCACAGCACACAAAGACC	80	47
112740	112759	388010	GAAGCCACAAAATCCACAGC	86	48
112784	112803	388011	GGTAGTCACTTAGGTGTTTT	49	49
112790	112809	388012	ATAAGTGGTAGTCACTTAGG	57	50
112796	112815	388013	TTAGAAATAAGTGGTAGTCA	57	51
112833	112852	388014	AACTTCTGAACAACAGCAAC	82	52
112862	112881	388015	CTTATAATATATGATAGCAA	34	53
112887	112906	388016	GTATCATTAAAAGACACCTA	86	54
112904	112923	388017	GTCATTATTCTTAGACAGTA	82	55
113074	113093	388018	TATTTTTGCAATGAGATAAC	28	56
113081	113100	388019	AATAAAATATTTTTGCAATG	0	57
113124	113143	388020	GCTTATAAGCATGATTTTTA	31	58
113134	113153	388021	AATTCATGTTGCTTATAAGC	51	59
113146	113165	388022	GTGTCAGTTCTTAATTCATG	20	60
113177	113196	388023	GGCTATTAATAACTTTATAT	29	61
113187	113206	388024	TTCTTCAAATGGCTATTAAT	45	62
113264	113283	388025	TTCTGGCAGTGTTGCTTCAG	59	63
113284	113303	388026	CAGTGCATACCAAAACACAC	61	64
113294	113313	388027	CTTAAGGAACCAGTGCATAC	77	65
113304	113323	388028	ATCACAGCCACTTAAGGAAC	31	66
113314	113333	388029	TCAATAATTAATCACAGCCA	70	67
113354	113373	388030	CCACTCTACAATAGTAGTTG	44	68
113525	113544	388031	TATCAGACAAAATAGATTTT	0	69
113535	113554	388032	TTCACACCAATATCAGACAA	67	70
113555	113574	388033	ATTGTCAGAAAGGTACAGCA	64	71
113565	113584	388034	AATATTATTTATTGTCAGAA	0	72
113573	113592	388035	CATGGTCGAATATTATTTAT	5	73
113002	113021	388037	TCGCAAAATGGTAAAATTTC	35	74
2053	2072	388039	GTCTGCGCTGCAGCCCGCAC	79	75

2183	2202	388040	GGAGGCAAACCCGCTAACCT	63	76
3590	3609	388042	GTTTACCTACCTACATAGAG	8	77
10952	10971	388043	GTTTTGGAGCCTACAAAAAC	56	78
16748	16767	388044	TTCTCAGCCACTGGTACAAA	40	79
49342	49361	388045	CCATTCCCAAGAGACCCAGA	92	80
73617	73636	388046	AGAAGAATCAATTGCTTTAC	85	81
94236	94255	388047	TAATCATTTAAACCTTAGTA	32	82
112476	112495	388048	GATACCCTTCCTAATATTAG	46	83

Table 3

Inhibition of human SNCA mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NOs: 3-7

Target SEQ ID NO	Start Site	Stop Site	Oligo ID	Sequence	% inhibition	SEQ ID NO
3	310	329	388036	GATACCCTTCCTTGCCCAAC	12	84
4	124	143	388038	GCCACTACATAGAGAACACC	78	85
5	392	411	388041	CCTTTACACCACACTGAGTC	91	86
6	595	614	388049	ATATCTGCCAGAATGTCCTT	86	87
7	62	81	388050	TTACACCACACTCACTTCCG	55	88

Example 2: Dose-dependent antisense inhibition of human SNCA in HuVEC cells

Eleven gapmers, exhibiting over 84 percent or greater *in vitro* inhibition of human SNCA in the study described in Example 1, were tested at various doses in HuVEC cells. Cells were plated at a density of 6,000 cells per well and transfected using LipofectAMINE2000® reagent with 0.08 nM, 0.25 nM, 0.74 nM, 2.22 nM, 6.67 nM, and 20.00 nM concentrations of antisense oligonucleotide, as specified in Table 4. After a treatment period of approximately 16 hours, RNA was isolated from the cells and SNCA mRNA levels were measured by quantitative real-time PCR. Human SNCA primer probe set RTS2621 (described herein above in Example 1) was used to measure mRNA levels. SNCA mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of SNCA, relative to untreated control cells. As illustrated in Table 4, SNCA mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 4

Dose-dependent antisense inhibition of human SNCA in HuVEC cells

Oligo ID	0.08 nM	0.25 nM	0.74 nM	2.22 nM	6.67 nM	20.00 nM	IC_{50} (nM)
387973	0	11	23	46	72	81	2.6
387975	9	8	25	57	72	83	2.1
387978	13	28	39	68	81	89	1.1
387983	0	8	17	49	75	85	2.6
387984	3	15	30	66	82	86	1.5
387985	0	6	24	66	77	89	1.8
387986	0	17	33	67	77	84	1.7
388004	0	11	30	65	78	86	1.8
388008	2	0	26	59	77	88	2.1
388010	0	8	24	54	71	87	2.3
388041	0	10	27	55	77	86	2.2

Example 3: Dose-dependent antisense inhibition of human SNCA in SH-SY5Y cells

Gapmers were selected from the study described in Example 2 and tested at various doses in SH-SY5Y cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 5 µM, 10 µM, and 20 µM concentrations of antisense oligonucleotide, as specified in Table 5. After a treatment period of approximately 16 hours, RNA was isolated from the cells and SNCA mRNA levels were measured by quantitative real-time PCR. Human SNCA primer probe set RTS2620 (forward sequence GGTGCTTCCCTTTCACTGAAGT, designated herein as SEQ ID NO: 89; reverse sequence ACATCGTAGATTGAAGCCACAAAA, designated herein as SEQ ID NO: 90, probe sequence AATACATGGTAGCAGGGTCTTTGTGTGCTGTG, designated herein as SEQ ID NO: 91) was used to measure mRNA levels. SNCA mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of SNCA, relative to untreated control cells. As illustrated in Table 5, SNCA mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 5

Dose-dependent antisense inhibition of human SNCA in SH-SY5Y cells

Oligo ID	5 µM	10 µM	20 µM
387978	79	85	94
387984	79	92	96
387985	54	82	93
387986	63	84	91
388004	71	88	92

Example 4: Tolerability of antisense oligonucleotides targeting human SNCA in a mouse model

ISIS oligonucleotides that demonstrated dose-dependent inhibition in the studies described herein in Examples 2 and 3 were evaluated for tolerability in a mouse model by monitoring changes in the levels of various metabolic markers in C57BL/6 mice.

Treatment

C57BL/6 mice were injected with 50 mg/kg of ISIS 387973, ISIS 387975, ISIS 387978, ISIS 387983, ISIS 387984, ISIS 387985, ISIS 387986, ISIS 388004, ISIS 388008, ISIS 388010, or ISIS 388041 administered subcutaneously twice a week for 3 weeks. A control group of mice was injected with phosphate buffered saline (PBS) administered subcutaneously twice a week for 3 weeks. Mice were sacrificed 48 hrs after receiving the last dose. Plasma was collected for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured at the end of the treatment period. The results presented in Table 6 indicate that liver transaminases were within normal parameters for all the ISIS oligonucleotides, except for ISIS 387986.

Table 6

Effect of antisense oligonucleotide treatment on ALT and AST (IU/L) of C57BL/6 mice

	ALT	AST
PBS	32	62
ISIS 387973	37	65
ISIS 387975	67	94
ISIS 387978	33	51
ISIS 387983	45	81
ISIS 387984	60	75
ISIS 387985	30	49
ISIS 387986	780	384
ISIS 388004	36	59

ISIS 388008	48	66
ISIS 388010	73	79
ISIS 388041	61	90

Body and organ weights

The body weights of the mice, as well as liver, spleen and kidney weights were measured at the end of the study. All the weights measured were within 13% that of the corresponding weights in the PBS control. The results demonstrate that none of the ISIS oligonucleotides had any adverse effect on the overall health of the mice.

Example 5: Potency of antisense oligonucleotides targeting human SNCA in a transgenic mouse model (SNCA PAC mice)

The ISIS oligonucleotides were further evaluated for potency in the SNCA PAC (PAC-Tg(SNCA^{WT}) Snca$^{-/-}$) transgenic mouse model. These mice harbor a knockout *Snca* allele and a transgene encoding human SNCA under a PAC (P1 artificial chromosome construct) promoter.

Treatment

Groups of 4 SNCA PAC mice each were injected with 100 µg of ISIS 387973, ISIS 387975, ISIS 387978, ISIS 387983, ISIS 387984, ISIS 387985, ISIS 388004, ISIS 388008, ISIS 388010, or ISIS 388041 administered via an intrastriatal bolus injection. A control group of mice was injected with phosphate buffered saline (PBS) administered via an intrastriatal bolus injection. Mice were sacrificed 2 weeks after receiving the injection. Brain tissue was collected for further analysis.

RNA analysis

RNA was extracted from the striatal and cortical tissues of the brain for real-time PCR analysis of human SNCA mRNA. The results are presented in Table 7, and demonstrate that most of the ISIS oligonucleotides inhibit human SNCA mRNA significantly compared to the PBS control.

Table 7

Percent inhibition of human SNCA mRNA in SNCA PAC mice compared to the PBS control

Oligo ID	Striatum	Cortex
387973	99	92
387975	93	65

387978	39	69
387983	97	65
387984	90	78
387985	98	75
388004	98	54
388008	0	0
388010	0	15
388041	99	74

Example 6: Antisense inhibition of human SNCA in SH-SY5Y cells by oligonucleotides designed by microwalk

Additional gapmers were designed targeting the region of the SNCA gene between the target sites of ISIS 387984 (start site 404 of SEQ ID NO: 1) and ISIS 387985 (start site 444 of SEQ ID NO: 1), which demonstrated significant inhibition of SNCA mRNA. These gapmers were designed by creating gapmers shifted by one nucleobase from each other (i.e. “microwalk”) of the region between the two gapmers. The new antisense oligonucleotides were designed as 5-10-5 gapmers. These gapmers were tested *in vitro*. ISIS 387984 and ISIS 387985 were also included in the assay for comparison. Cultured SH-SY5Y cells at a density of 5,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and SNCA mRNA levels were measured by quantitative real-time PCR. Two human primer probe set 672 (forward sequence TGGCAGAAGCAGCAGGAAA, designated herein as SEQ ID NO: 95; reverse sequence TCCTTGGTTTTGGAGCCTACA, designated herein as SEQ ID NO: 96; probe sequence CAAAAGAGGGTGTTCTC, designated herein as SEQ ID NO: 97) and primer probe set 673 (forward sequence GGAGCAGGGAGCATTGCA, designated herein as SEQ ID NO: 92; reverse sequence CCTTCTTCATTCTTGCCCAACT, designated herein as SEQ ID NO: 93; probe sequence CACTGGCTTTGTCAAAA, designated herein as SEQ ID NO: 94) were individually used to measure SNCA mRNA levels. SNCA mRNA levels were adjusted according to total RNA content, as measured by Cyclophilin levels. Results are presented as percent inhibition of SNCA, relative to untreated control cells. The results are presented in Table 8.

The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of ten 2’-deoxynucleotides and is flanked on both sides (in the 5’ and 3’ directions) by wings comprising five nucleosides each. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. “Target start site” indicates the 5’-most nucleoside to which the gapmer is targeted. “Target stop site” indicates the 3’-most nucleoside to which the gapmer is targeted. Each gapmer listed in Table 8 is targeted SEQ ID NO: 1 (GENBANK Accession No. NM_000345.3).

As shown in Table 8, several of the gapmers exhibited at least 50% inhibition, as measured by primer probe set 673, including ISIS numbers: 387984, 489351, 489352, 489353, 489354, 489355, 489356, 489357, 489358, 489359, 489360, 489361, 489362, 489364, 489365, 489366, 489367, 489368, 489369, 489371, 489372, 489373, 489374, 489375, 489381, 489382, 489383, 489387, and 387985.

Several of the gapmers exhibited at least 60% inhibition, including ISIS numbers: 387984, 489351, 489352, 489353, 489355, 489356, 489357, 489358, 489359, 489360, 489361, 489366, 489371, 489372, 489373, 489374, 489381, 489383, and 387985.

Several of the gapmers exhibited at least 70% inhibition, including ISIS numbers: 387984, 489351, 489352, 489356, 489357, 489358, 489359, 489360, 489361, 489373, 489374, 489381, and 387985.

Several of the gapmers exhibited at least 80% inhibition, including ISIS numbers: 489357, 489358, 489359, and 489360.

Two of the gapmers exhibited at least 85% inhibition, including ISIS numbers: 489357 and 489358.

One gapmer exhibited at least 90% inhibition, which is ISIS 489357.

Table 8

Inhibition of human SNCA mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

Target Start Site	Target Stop Site	Oligo ID	Sequence	% inhibition (primer probe set 672)	% inhibition (primer probe set 673)	SEQ ID NO
404	423	387984	TTGCCACACCATGCACCACT	79	76	22
405	424	489351	GTTGCCACACCATGCACCAC	81	76	98
406	425	489352	TGTTGCCACACCATGCACCA	75	70	99
407	426	489353	CTGTTGCCACACCATGCACC	70	64	100
408	427	489354	ACTGTTGCCACACCATGCAC	62	56	101
409	428	489355	CACTGTTGCCACACCATGCA	67	61	102
410	429	489356	CCACTGTTGCCACACCATGC	82	79	103

411	430	489357	GCCACTGTTGCCACACCATG	92	90	104
412	431	489358	AGCCACTGTTGCCACACCAT	90	87	105
413	432	489359	CAGCCACTGTTGCCACACCA	89	83	106
414	433	489360	TCAGCCACTGTTGCCACACC	88	84	107
415	434	489361	CTCAGCCACTGTTGCCACAC	83	76	108
416	435	489362	TCTCAGCCACTGTTGCCACA	64	57	109
417	436	489363	TTCTCAGCCACTGTTGCCAC	54	49	110
418	437	489364	CTTCTCAGCCACTGTTGCCA	65	59	111
419	438	489365	TCTTCTCAGCCACTGTTGCC	58	53	112
420	439	489366	GTCTTCTCAGCCACTGTTGC	68	64	113
421	440	489367	GGTCTTCTCAGCCACTGTTG	62	51	114
422	441	489368	TGGTCTTCTCAGCCACTGTT	61	54	115
423	442	489369	TTGGTCTTCTCAGCCACTGT	61	53	116
424	443	489370	TTTGGTCTTCTCAGCCACTG	55	49	117
425	444	489371	CTTTGGTCTTCTCAGCCACT	75	68	118
426	445	489372	TCTTTGGTCTTCTCAGCCAC	65	60	119
427	446	489373	CTCTTTGGTCTTCTCAGCCA	79	75	120
428	447	489374	GCTCTTTGGTCTTCTCAGCC	76	72	121
429	448	489375	TGCTCTTTGGTCTTCTCAGC	58	51	122
430	449	489376	TTGCTCTTTGGTCTTCTCAG	46	38	123
431	450	489377	CTTGCTCTTTGGTCTTCTCA	49	46	124
432	451	489378	ACTTGCTCTTTGGTCTTCTC	44	34	125
433	452	489379	CACTTGCTCTTTGGTCTTCT	46	35	126
434	453	489380	TCACTTGCTCTTTGGTCTTC	50	45	127
435	454	489381	GTCACTTGCTCTTTGGTCTT	80	73	128
436	455	489382	TGTCACTTGCTCTTTGGTCT	67	58	129
437	456	489383	TTGTCACTTGCTCTTTGGTC	70	65	130
438	457	489384	TTTGTCACTTGCTCTTTGGT	42	31	131
439	458	489385	ATTTGTCACTTGCTCTTTGG	54	43	132
440	459	489386	CATTTGTCACTTGCTCTTTG	42	38	133
441	460	489387	ACATTTGTCACTTGCTCTTT	58	50	134
442	461	489388	AACATTTGTCACTTGCTCTT	46	39	135
443	462	489389	CAACATTTGTCACTTGCTCT	59	49	136
444	463	387985	CCAACATTTGTCACTTGCTC	76	71	23

Example 7: Potency of antisense oligonucleotides targeting human SNCA in a transgenic mouse model (SNCA PAC mice)

The ISIS oligonucleotides that demonstrated significant inhibition in the study described herein in Example 6 were further evaluated for potency in SNCA PAC mice.

Treatment

Groups of 12 SNCA PAC mice each were injected with 50 μg of ISIS 387985, ISIS 489351, ISIS 489352, ISIS 489356, ISIS 489357, ISIS 489358, ISIS 489359, ISIS 489360, ISIS 489373, ISIS 489374, ISIS 489381, or ISIS 489383 administered via an intrastriatal bolus injection. A control group of mice was injected with phosphate buffered saline (PBS) administered via an intrastriatal bolus injection. Mice were sacrificed 2 weeks after receiving the injection. Brain tissue was collected for further analysis.

RNA analysis

RNA was extracted from the hippocampal, striatal and cortical tissues of the brain for real-time PCR analysis of human SNCA mRNA using primer probe set 673 (described herein in Example 6 above). The results are presented in Table 9, and demonstrate that most of the ISIS oligonucleotides inhibit human SNCA mRNA significantly compared to the PBS control.

Table 9

Percent (%) inhibition of human SNCA mRNA in SNCA PAC mice compared to the PBS control

Oligo ID	Cortex	Striatum	Hippocampus
387985	86	76	72
489351	77	31	28
489352	81	38	54
489356	83	0	43
489357	91	49	76
489358	75	0	76
489359	81	62	65
489360	72	0	70
489373	78	34	64
489374	77	53	82
489381	73	34	72
489383	59	61	34

Example 8: Potency of antisense oligonucleotides targeting human SNCA in a transgenic mouse model (Thy1-aSYN mice)

The ISIS oligonucleotides that demonstrated significant inhibition in the study described herein in Example 7 were further evaluated in Thy1-aSYN mice.

Treatment

Groups of 4 Thy1-aSYN mice each were injected with 50 µg of ISIS 387985, ISIS 489352, ISIS 489356, and ISIS 489357 administered via an intrastriatal bolus injection. Mice were anesthetized with sodium pentobarbitone (66 mg/kg Nembutal in sterile 0.9% saline, i.p.). The scalps of the mice were then shaved and, following loss of the pedal reflex, mice were placed in a stereotaxic frame (David Kopf Instruments, CA). To maintain a surgical plane of anesthesia, mice were administered with isoflurane (1-2% in 100% oxygen at 0.5L/min) via a nose cone, as required. The scalp was sterilized using three alternating wipes of Betadine and 70% ethanol. An incision was made in the scalp and the skull surface exposed and bregma positively identified. A hole was drilled in the skull at 0.5 mm AP, 2mm ML, relative to bregma. ISIS 387985, ISIS 489352, ISIS 489356, and ISIS 489357 at a dose of 50 µg in a 2 µL solution was injected unilaterally into the right striatum, using a 10uL Hamilton syringe with a 27 gauge needle connected to a microsyringe pump controller (KD Scientific 310) at a flow rate of 0.2uL/min. The DV coordinate was measured at 3 mm below the skull surface. The needle was left in place for a further 3 minutes after injection to allow diffusion of the solution into the brain. After slowly withdrawing the syringe, the scalp was sutured and mice were subcutaneously injected with 0.5 mL warm sterile saline to aid rehydration, and placed on a warm water heat pad and monitored until they regained consciousness and mobility. A group of 4 mice was injected with PBS in a similar manner. Mice were returned to their home cage and supplied with mashed food on the cage floor. The body weights and health of mice was monitored daily post-surgery. Mice were sacrificed 2 weeks after receiving the injection. Brain tissue was collected for further analysis. A group of 4 mice was injected with PBS in a similar manner.

RNA analysis

RNA was extracted from the striatal and cortical tissues of the brain for real-time PCR analysis of human SNCA mRNA normalized to Cyclophilin A mRNA. The results are presented in Table 10.

Table 10

Percent inhibition of human SNCA mRNA in Thy1-aSYN mice compared to the PBS control

Oligo ID	Cortex	Striatum
387985	67	63

489352	50	18
489356	56	20
489357	64	53

Protein analysis

Protein was extracted from cell lysates of the striatal and cortical tissues of the brain and quantified by western blot analysis using anti-alpha-synuclein, clone Syn211 (Millipore, NY). The results were normalized to alpha-tubulin and are presented in Table 11.

Table 11

Percent inhibition of human SNCA protein levels in Thy1-aSYN mice compared to the PBS control

Oligo ID	Cortex	Striatum
387985	24	37
489352	30	51
489356	0	66
489357	0	78

Quantification of antisense oligonucleotide levels in brain sections

The rostral and caudal regions of striatal and cortical tissues of the brain were individually stained using immunofluoresent antibodies against the antisense oligonucleotides (Ab6653, ISIS Pharmaceuticals, CA) or mouse anti-SNCA (BD Transduction Laboratories, CA). Images of the stained sections were acquired using a microarray scanner (Agilent Technologies, CA). Immunofluorescent intensity was quantified using ImageJ (NIH). The results of the quantification of immunofluoresence are presented in Tables 12 and 13. The results from Table 12 demonstrate the even distribution of the antisense oligonucleotides to different regions of the brain, relative to the PBS control level, which was designated zero intensity. Table 13 presents the SNCA protein levels in the corresponding brain sections, and demonstrates inhibition of SNCA by some of the ISIS oligonucleotides.

Table 12

Antisense oligonucleotide levels in Thy1-aSYN mice compared to the PBS control (arbitrary units)

Oligo ID	Cortex (rostral)	Striatum (rostral)	Cortex (caudal)	Striatum (caudal)
387985	22607	25225	29899	34625

489352	34604	30315	32535	36067
489356	26615	22943	26549	24441
489357	25219	25095	27427	30458

Table 13

Percent reduction in SNCA levels in Thy1-aSYN mice compared to the PBS control

Oligo ID	Cortex (rostral)	Striatum (rostral)	Cortex (caudal)	Striatum (caudal)
387985	17	23	37	16
489352	14	12	28	10
489356	0	0	0	0
489357	0	0	21	0

Evaluation of toxicity due to antisense oligonucleotide administration in brain sections

The rostral and caudal regions of striatal and cortical tissues of the brain were also individually stained with immunofluorescent antibodies rabbit anti-GFAP (Dako Inc, CA) or anti-NeuN (Chemicon Inc). Images of the stained sections were acquired using a microarray scanner (Agilent Technologies, CA). Immunofluorescent intensity was quantified using ImageJ (NIH). The results of the quantification are presented in Tables 14 and 15. Table 14 shows the levels of glial fibrillary acidic protein (GFAP), which is moderately increased in a non-specific manner as a result of antisense oligonucleotide administration. This is an expected outcome (Chiasson et al., *Cell. Mol. Neurobiol.* 1994. 14: 507-521) and the results demonstrate that the increase is non-significant. Table 15 presents the data on NeuN, a neuron marker that indicates neuronal toxicity. The results indicate none of the ISIS oligonucleotides induced increase in NeuN levels relative to the PBS control.

The brain sections were separately stained with rabbit anti-Iba1 (Wako Chem. Inc, CA) to detect microglial cells, followed by probing with a biotinylated secondary antibody. The sections were developed using a complex of avidin-biotin peroxidase. The sections were then developed by DAB substrate. The optical fractionator function of Stereo Investigator (MicroBrightField) was used to count 4 representative samples of Iba1-positive microglial cells in the striatum and cortex. The microglia were then scored as either resting or activated microglia. The scoring was based on morphological criteria of either ramified (resting) or amoeboid (activated) appearance. Activated microglia are a marker of neuronal toxicity. The average of the results was expressed as a percent of the number of activated Iba1-positive cells compared to the total number of Iba1-positive cells. The results are presented in Table 18, and demonstrate that treatment with either ISIS 387985 or ISIS 489357 does not cause microglial activation. Hence, treatment with either antisense oligonucleotide did not cause any neural toxicity.

Table 14

Percent increase in GFAP levels in Thy1-aSYN mice compared to the PBS control

Oligo ID	Cortex (caudal)	Striatum (caudal)
387985	70	128
489352	66	151
489356	61	82
489357	120	130

Table 15

Percent change in NeuN levels in Thy1-aSYN mice compared to the PBS control

Oligo ID	Cortex (caudal)	Striatum (caudal)
387985	-11	-11
489352	-28	-38
489356	-5	-1
489357	-10	-15

Table 16

Percent of activated microglia in Thy1-aSYN mice

	Cortex	Striatum
PBS	7	19
ISIS 387985	26	27
ISIS 489352	43	49
ISIS 489356	35	66
ISIS 489357	21	37

Example 9: Potency of antisense oligonucleotides targeting human SNCA in a transgenic mouse model (Thy1-aSYN mice)

Some of the ISIS oligonucleotides from the study described herein in Example 5 were further evaluated in Thy1-aSYN mice, which overexpress human SNCA (Rockenstein et al., J.

Neurosci. Res. 68: 568-578, 2002). ISIS 387978, ISIS 387983, ISIS 387984, and ISIS 387985 all target the transgene mRNA in Thy-aSYN mice and were tested in this model.

The target sites of the human oligonucleotides to the human mRNA sequence, SEQ ID NO: 1 (GENBANK Accession No. NM_000345.3) are presented in Table 17. Some of the human oligonucleotides are cross-reactive with mouse SNCA sequences. The greater the complementarity between the human oligonucleotide and the murine sequence, the more likely the human oligonucleotide can cross-react with the murine sequence. The target start sites of the human oligonucleotides to the murine sequence SEQ ID NO: 137 (GENBANK Accession No NM_001042451.1) are also presented in Table 17. 'n/a' indicates that the antisense oligonucleotide has more than 3 mismatches to the murine sequence.

Table 17

Target Start Sites of antisense oligonucleotides targeting SEQ ID NO: 1 and SEQ ID NO: 137

Human Target Start Site	ISIS No	Murine Target Start Site	SEQ ID NO
282	387978	318	16
381	387983	n/a	20
404	387984	n/a	22
444	387985	480	23

Treatment

Groups of 4 Thyl-aSYN mice each were injected with 50 µg of ISIS 387978, ISIS 387983, ISIS 387984, or ISIS 387985, administered via intrastriatal bolus injection. Mice were anesthetized with sodium pentobarbitone (66 mg/kg Nembutal in sterile 0.9% saline, i.p.). The scalps of the mice were then shaved and, following loss of the pedal reflex, mice were placed in a stereotaxic frame (David Kopf Instruments, CA). To maintain a surgical plane of anesthesia, mice were administered with isoflurane (1-2% in 100% oxygen at 0.5 L/min) via a nose cone, as required. Oxygen was administered throughout the surgery and for 30 min post-surgically. The temperature of the mice was monitored using a rectal probe thermometer (Physitemp). The scalp was sterilized using three alternating wipes of Betadine and 70% ethanol. An incision was made in the scalp and the skull surface exposed and bregma positively identified. After ensuring that the skull surface was flat, i.e. a dorsoventral (DV) deviation of <0.2 mm at bregma +/- 2 mm antero-posterior (AP), a hole was drilled in the skull at 0.5 mm AP, 2mm medialateral (ML), relative to bregma. Each of the ISIS oligonucleotides at a concentration of 50 mg/mL in a 2 μL solution was injected unilaterally into the right striatum, using a 10 μL Hamilton syringe with a 27 gauge needle connected to a microsyringe pump controller (KD Scientific 310) at a flow rate of 0.2 μL/min. The DV coordinate was measured at 3 mm below the skull surface. The needle was left in place for a further 3 minutes after injection to allow diffusion of the solution into the brain. After slowly withdrawing the syringe, the scalp was sutured, and the mice were subcutaneously injected with 0.5 mL warm sterile PBS, to aid rehydration. The mice were placed on a warm water heat pad and monitored until they regained consciousness and mobility. A group of 4 mice was injected with PBS in a similar manner. The animals were then returned to their home cage and supplied with mashed food on the cage floor. The body weights and health of mice was monitored daily post-surgery. Mice were sacrificed 2 weeks after receiving the injection by cervical dislocation.

The brains of the mice were immediately collected and dissected. Using a coronal brain matrix, 1 mm slices of the brain were harvested for mRNA and protein extraction. A 1 mm slice immediately rostral to the injection site was taken for mRNA and a 1 mm slice immediately caudal to the injection site was taken for protein analyses. The striatum and cortex from the ipsilateral hemisphere were dissected on ice.

RNA analysis

For mRNA purification, brain tissue was rapidly frozen on dry ice in 2 mL tubes containing 0.5 mL GITC/BME and sterile ceramic beads. RNA was extracted from the striatal and cortical tissues of the brain for real-time PCR analysis of human SNCA mRNA normalized to Cyclophilin A mRNA. Human SNCA mRNA levels were measured using human primer probe set RTS2618 (forward sequence AGACCAAAGAGCAAGTGACAAATG, designated herein as SEQ ID NO: 138; reverse sequence CCTCCACTGTCTTCTGGGCTACT, designated herein as SEQ ID NO: 139; probe sequence TGGAGGAGCAGTGGTGACGGGTG, designated as SEQ ID NO: 140). The results are presented in Table 18, expressed as percent inhibition compared to the PBS control. Mouse SNCA mRNA levels were also measured using murine primer probe set RTS2956 (forward sequence GTCATTGCACCCAATCTCCTAAG, designated herein as SEQ ID NO: 141; reverse sequence GACTGGGCACATTGGAACTGA, designated herein as SEQ ID NO: 142; probe sequence CGGCTGCTCTTCCATGGCGTACAA, designated herein as SEQ ID NO: 143). The results are presented in Table 19, expressed as percent inhibition compared to the PBS control. Since

ISIS 387978 and ISIS 387985 both target SEQ ID NO: 137, treatment with either antisense oligonucleotide inhibits murine SNCA mRNA expression.

Table 18

Percent inhibition of human SNCA mRNA in Thy1-aSYN mice compared to the PBS control

ISIS No	Striatum	Cortex
387978	35	0
387983	16	0
387984	67	35
387985	89	70

Table 19

Percent inhibition of murine SNCA mRNA in Thy1-aSYN mice compared to the PBS control

ISIS No	Striatum	Cortex
387978	62	44
387983	16	0
387984	18	2
387985	84	83

Protein analysis

Tissue samples for protein analysis were rapidly frozen in tubes containing sterile ceramic beads. Protein levels of SNCA were measured by western blot analysis using an anti-SNCA antibody (Signet, #4D6) targeting both human and murine SNCA. The results are presented in Table 20, expressed as percent inhibition compared to the PBS control.

Table 20

Percent inhibition of SNCA protein levels in Thy1-aSYN mice compared to the PBS control

ISIS No	Striatum	Cortex
387978	0	0
387983	9	0
387984	0	0
387985	29	76

Immunofluorescence analysis

One coronal section from each brain was taken at the level of the caudal striatum. After washing in PBS, the sections were incubated in M.O.M. mouse IgG blocking reagent (Vector Laboratories, PK-2200) for 1 hour. Sections were then incubated overnight at 4°C in 2% NGS, 0.5% Triton X-100 in PBS with primary antibodies, mouse anti-NeuN (1: 500 dilution; Chemicon MAB377) and 6653Ab rabbit anti-ASO (1: 3,000 dilution; ISIS Pharmaceuticals). After washing in PBS, the sections were incubated for 2 hours in 5% NGS in PBS with secondary antibodies, Cy3-conjugated goat anti-rabbit (1: 250 dilution; Millipore) and Cy5-conjugated goat anti-mouse (1: 250 dilution; Jackson Immunoresearch). Several sections were incubated with secondary antibodies alone, omitting primary antibody incubation, to serve as controls. After washing in PBS, sections were mounted onto glass microscope slides in water and dried overnight. Slides were scanned using a high-resolution microarray scanner (Agilent) using lasers to excite the Cy3 and Cy5 fluorochromes. The images of the scanned sections were then analyzed using ImageJ (NIH) to quantify the intensity of the immunofluorescent staining. The average intensity of staining in the striatum and cortex of the ipsilateral and contralateral hemispheres from the brains of mice receiving ASOs was calculated and compared to that of the control mice. The immunofluorescence intensity of the PBS control was considered the baseline and was arbitrarily designated as 1.00.The results are presented in Table 21 and indicate that there was negligible neuronal toxicity in most of the ISIS oligonucleotides tested.

Table 21

NeuN quantification by immunofluorescent intensity in the striatum and cortex

	Striatum	Cortex
PBS	1.00	1.00
387978	0.47	0.85
387983	0.77	1.17
387984	0.78	1.02
387985	0.90	0.96

The distribution of ASO, as displayed by Ab6653 staining, was widespread throughout the ipsilateral hemisphere, including the striatum and cortex, extending along the entire rostral-caudal axis of the striatum. Other brain structures, including the globus pallidus, the rostral extent of the hippocampus and the thalamus, were also immunopositive.

Example 10: Effect on behavior of Thy1-aSYN mice after administration of antisense oligonucleotides targeting human SNCA

ISIS 387985, which demonstrated significant potency in the studies described above is administered to Thy1-aSYN mice. Motor function, olfaction, and spatial memory are tested in the mice.

Treatment

Groups of 16 male Thy1-aSYN mice each, 3.5 months in age, are infused ICV, using Alzet minipump model #2002 with brain infusion kit, with 50 µg/day of ISIS 387985 or with sterile PBS for 2 weeks. This is followed by 2 weeks washout, wherein the minipump is removed and mice are allowed to recover. The mice are tested behaviorally between 4.5 months and 5 months of age. The tests used to analyze behavior are a motor test, which includes a challenging beam and pole task (Fleming, S.M. et al., J Neurosci. 24: 9434-9440, 2004), an olfaction test using a buried pellet (Fleming, S.M. et al., Eur. J. Neurosci. 28: 247-256, 2008), and a spatial working memory test using novel place recognition (Magen et al., submitted). Mice are euthanized at 5 months of age. The brain and peripheral tissues are harvested for biochemical and histological analysis.

Embodiments include:

1. A modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: 11 to 88 and 98 to 136.

2. The modified oligonucleotide of embodiment 1, wherein the modified oligonucleotide is a single-stranded oligonucleotide.

3. The modified oligonucleotide of embodiment 2, wherein the modified oligonucleotide has a nucleobase sequence that is 100% complementary to a human alpha-synuclein nucleic acid.

4. The modified oligonucleotide of embodiment 2, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

5. The modified oligonucleotide of embodiment 4, wherein at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.

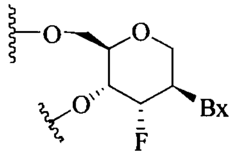
6. The modified oligonucleotide of embodiment 2, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar.

7. The modified oligonucleotide of embodiment 6, wherein the modified sugar is a bicyclic sugar.

8. The modified oligonucleotide of embodiment 7, wherein the bicyclic sugar comprises a 4'-CH(CH_3)-O-2' bridge.

9. The modified oligonucleotide of embodiment 6, comprising at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

10. The modified oligonucleotide of embodiment 9, wherein each of the at least one tetrahydropyran modified nucleoside has the structure:



wherein Bx is an optionally protected heterocyclic base moiety.

11. The modified oligonucleotide of embodiment 6, wherein the modified sugar comprises a 2'-O-methoxyethyl group.

12. The modified oligonucleotide of embodiment 2, wherein at least one nucleoside of the modified oligonucleotide comprises a modified nucleobase.

13. The modified oligonucleotide of embodiment 12, wherein the modified nucleobase is a 5-methylcytosine.

14. The modified oligonucleotide of embodiment 2, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxy nucleosides;

a 5' wing segment consisting of linked nucleosides;

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.

15. The modified oligonucleotide of embodiment 14, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;
a 5' wing segment consisting of five linked nucleosides;
a 3' wing segment consisting of five linked nucleosides;

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

16. A method, comprising identifying an animal having a neurodegenerative disease and administering to said animal a therapeutically effective amount of a composition comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NOs: SEQ ID NOs: 11 to 88 and 98 to 136.

17. The method of embodiment 16, wherein said administration reduces expression of alpha-synuclein.

18. The method of embodiment 16, wherein said administration improves motor coordination.

19. The method of embodiment 16, wherein said administration improves olfaction.

20. The method of embodiment 16, wherein said administration improves spatial memory.

21. The method of embodiment 16, wherein said administration reduces aggregation of alpha-synuclein.

22. A modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 404 to 463 of SEQ ID NO: 1; and wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

23. The modified oligonucleotide of embodiment 22, wherein said modified oligonucleotide consists of a nucleobase sequence selected from the group consisting of SEQ ID NO: 23, 98, 99, 103, 104, 105, 106, 107, 120, and 121.

24. The modified oligonucleotide of embodiment 22, wherein said modified oligonucleotide consists of ISIS 387985, ISIS 489351, ISIS 489352, ISIS 489356, ISIS 489357, ISIS 389358, ISIS 489359, ISIS 489360, ISIS 489373, and ISIS 489374.

Claims:

1. A modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of a nucleobase sequence selected from among SEQ ID NOs: 11 to 88 and 98 to 136.

2. A modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases 100% complementary to an equal length portion of nucleobases:

(i) 236-301 of SEQ ID NO: 1
(ii) 304-331 of SEQ ID NO: 1;
(iii) 107-126 of SEQ ID NO: 1;
(iv) 361-380 of SEQ ID NO: 1;
(v) 375-400 of SEQ ID NO: 1;
(vi) 404-463 of SEQ ID NO: 1;
(vii) 469-488 of SEQ ID NO: 1;
(viii) 542-573 of SEQ ID NO: 1;
(ix) 607-721 of SEQ ID NO: 1;
(x) 734-837 of SEQ ID NO: 1;
(xi) 881-927 of SEQ ID NO: 1;
(xii) 952-983 of SEQ ID NO: 1;
(xiii) 1001-1020 of SEQ ID NO: 1;
(xiv) 1030-1049 of SEQ ID NO: 1;
(xv) 1055-1074 of SEQ ID NO: 1;
(xvi) 1170-1189 of SEQ ID NO: 1;
(xvii) 1242-1268 of SEQ ID NO: 1;
(xviii) 1292-1333 of SEQ ID NO: 1;
(xix) 1345-1374 of SEQ ID NO: 1;
(xx) 1432-1501 of SEQ ID NO: 1;
(xxi) 1522-1541 of SEQ ID NO: 1;
(xxii) 1693-1760 of SEQ ID NO: 1;
(xxiii) 2183-2202 of SEQ ID NO: 2;
(xxiv) 3576-3609 of SEQ ID NO: 2;
(xxv) 16748-16794 of SEQ ID NO: 2;
(xxvi) 49342-49361 of SEQ ID NO: 2;
(xxvii) 73617-73636 of SEQ ID NO: 2;

(xxviii) 94236-94255 of SEQ ID NO: 2; or
(xxix) 112476-112553 of SEQ ID NO: 2.

3. The modified oligonucleotide of claim 1 or claim 2, wherein the modified oligonucleotide has a nucleobase sequence that is at least 90% complementary to a human alpha-synuclein nucleic acid.

4. The modified oligonucleotide of claim 1 or claim 2, wherein the modified oligonucleotide has a nucleobase sequence that is 100% complementary to a human alpha-synuclein nucleic acid.

5. The modified oligonucleotide of claim 3 or claim 4, wherein the human alpha-synuclein nucleic acid is SEQ ID NO: 1 or SEQ ID NO: 2.

6. The modified oligonucleotide of any one of claims 1-5, wherein the modified oligonucleotide is a single-stranded modified oligonucleotide.

7. The modified oligonucleotide of any one of claims 1-6, wherein at least one internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.

8. The modified oligonucleotide of claim 7, wherein each internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.

9. The modified oligonucleotide of claim 7 or claim 8, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

10. The modified oligonucleotide of any one of claims 1-9, wherein at least one internucleoside linkage of the modified oligonucleotide is a phosphodiester internucleoside linkage.

11. The modified oligonucleotide of any one of claims 1-10, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar.

12. The modified oligonucleotide of claim 11, wherein the modified sugar is a bicyclic sugar.

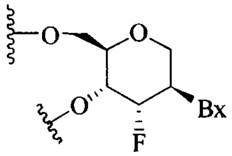
13. The modified oligonucleotide of claim 12, wherein the bicyclic sugar comprises a chemical bridge between the 4' and 2' positions of the sugar, wherein the chemical bridge is independently selected from: 4'-CH(R)-O-2' and 4'-$(CH_2)2$-O-2', wherein R is independently selected from C_1-C_6 alkyl and C_1-C_6 alkoxy.

14. The modified oligonucleotide of claim 13, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is independently selected from methyl, H, and $-CH_2$-O-CH_3.

15. The modified oligonucleotide of claim 11, wherein the modified sugar comprises a 2'-O-methoxyethyl group or a 2'-O-methyl group.

16. The modified oligonucleotide of any one of claims 1-15, comprising at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

17. The modified oligonucleotide of claim 16, wherein each of the at least one tetrahydropyran modified nucleoside has the structure:



wherein Bx is an optionally protected heterocyclic base moiety.

18. The modified oligonucleotide of any one of claims 1-17, wherein at least one nucleobase of the modified oligonucleotide is a modified nucleobase.

19. The modified oligonucleotide of claim 18, wherein the modified nucleobase is a 5-methylcytosine.

20. The modified oligonucleotide of any one of claims 1-19, wherein the modified oligonucleotide is a gapmer.

21. The modified oligonucleotide of claim 20, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxy nucleosides;
a 5' wing segment consisting of linked nucleosides;
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.

22. The modified oligonucleotide of claim 21, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;
a 5' wing segment consisting of five linked nucleosides;
a 3' wing segment consisting of five linked nucleosides;

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

23. A pharmaceutical composition comprising the modified oligonucleotide of any one of claims 1-22 and a pharmaceutically acceptable carrier or diluent.

24. The pharmaceutical composition pf claim 23, wherein the pharmaceutically acceptable diluent is phosphate-buffered saline (PBS).

25. Use of the modified oligonucleotide according to any one of claims 1-22 in the manufacture of a medicament for treating a human having a neurodegenerative disease.

26. The use according to claim 25, wherein said use reduces expression of alpha-synuclein and/or reduces aggregation of alpha-synuclein.

27. The use according to claim 25, wherein said use improves motor coordination.

28. The use according to claim 25, wherein said use improves olfaction.

29. The use according to claim 25, wherein said use improves spatial memory.

30. The modified oligonucleotide according to any one of claims 1-22 for use in treating a human having a neurodegenerative disease

31. The modified oligonucleotide for use according to claim 30, wherein said use reduces expression of alpha-synuclein and/or reduces aggregation of alpha-synuclein.

32. The modified oligonucleotide for use according to claim 30, wherein said use improves motor coordination.

33. The modified oligonucleotide for use according to claim 30, wherein said use improves olfaction.

34. The modified oligonucleotide for use according to claim 30, wherein said use improves spatial memory.

35. Use of the single-stranded modified oligonucleotide according to any one of claims 1-22 for treating a human having a neurodegenerative disease.

36. The use according to claim 35, wherein said use reduces expression of alpha-synuclein and/or reduces aggregation of alpha-synuclein.

37. The use according to claim 35, wherein said use improves motor coordination.

38. The use according to claim 35, wherein said use improves olfaction.

39. The use according to claim 35, wherein said use improves spatial memory.

40. A method.