

Compositions and Methods for Modulating Alpha-Synuclein Expression

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/618,693, filed January 8, 2024, which is incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on January 7, 2025, is named DNL-047-01-WO_SeqListing.XML and is 152 kb in size.

BACKGROUND

[0003] Neurodegenerative diseases, including Parkinson's disease (PD), pose a significant and growing health challenge globally. PD is a complex progressive neurodegenerative disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra, leading to motor dysfunction, tremors, rigidity, and postural instability. The exact etiology of PD remains elusive, but increasing evidence suggests that aberrant aggregation and accumulation of alpha-synuclein protein play a pivotal role in the pathogenesis of the disease.

[0004] Alpha-synuclein (aSyn or SNCA) is intricately involved in the regulation of synaptic function and neurotransmitter release. However, under pathological conditions, SNCA undergoes misfolding, aggregation, and forms insoluble fibrils known as Lewy bodies, which are the hallmark neuropathological features of PD and other neurodegenerative disorders such as multiple system atrophy (MSA), Lewy body dementia, Pure Autonomic Failure (PAF), and REM sleep Behavior Disorder (RBD) (collectively synucleinopathies). The abnormal aggregation of SNCA is believed to contribute to neuronal dysfunction and cell death, ultimately leading to the clinical manifestations observed in affected individuals.

[0005] Current therapeutic strategies for PD primarily focus on alleviating symptoms and enhancing dopaminergic neurotransmission; however, these approaches do not address the underlying mechanisms driving disease progression. There is, therefore, a pressing need for innovative therapeutic interventions that directly target the pathological processes associated with SNCA and its aggregation.

SUMMARY

[0006] Certain embodiments provide SNCA antisense oligonucleotides (SNCA ASOs) comprising nucleic acid sequences that comprises at least 9 contiguous nucleobases of a sequence selected from any one of SEQ ID NOs: 1-15 and 18-23 and at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, the SNCA ASOs are 10-25 nucleotides in length.

[0007] Certain embodiments provide SNCA ASOs comprising the nucleobase sequence of any one of SEQ ID NOs: 24-46 and at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, the SNCA ASOs are 10-25 nucleotides in length.

[0008] Certain embodiments provide SNCA ASOs comprising a nucleic acid sequence as set forth in any one of SEQ ID Nos: 1-15 and 18-23 that comprises at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, the SNCA ASOs are 10-25 nucleotides in length.

[0009] Certain embodiments provide SNCA ASOs 16-20 nucleotides in length comprising the nucleobase sequence of any one of SEQ ID NOs: 24-38 and 41-46 and at least one modified internucleoside linkage and/or at least one modified sugar.

[0010] Certain embodiments provide SNCA ASOs 18-20 nucleotides in length comprising the nucleobase sequence of any one of SEQ ID NOs: 1-15, 18-38, and 41-46 and at least one modified internucleoside linkage and/or at least one modified sugar.

[0011] Certain embodiments provide SNCA ASOs 16 nucleotides in length comprising the nucleobase sequence of any one of SEQ ID NOs: 24-38 and 41—46 and at least one modified internucleoside linkage and/or at least one modified sugar.

[0012] Certain embodiments provide SNCA ASO gapmers comprising a gap segment having the nucleobase sequence of any one of SEQ ID NOs: 24-46 and at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, the SNCA ASO gapmers are 16 nucleotides in length wherein the 5' and 3' wing segments are each 3 nucleotides in length.

[0013] In some embodiments, an SNCA ASO is conjugated to a targeting ligand to form a conjugate. The targeting ligand can be, but is not limited to, a molecule that specifically binds to the transferrin receptor (TfR) or a molecule expressed on the luminal surface of the blood brain barrier. The molecule can be, but is not limited to, an anti-TfR antibody or a TfR binding fragment thereof, or a Fc polypeptide modified to bind the TfR (WO2023279099, incorporated herein by reference).

[0014] Certain embodiments provide pharmaceutical compositions comprising any of the SNCA ASOs as described herein or a conjugate comprising a SNCA ASO as described herein and a pharmaceutically acceptable carrier or diluent.

[0015] Certain embodiments provide a method of generating a neuronal cell with decreased alpha-synuclein expression, the method comprising delivering to the neuron cell a SNCA ASO as described herein or a conjugate comprising a SNCA ASO as described herein, wherein the SNCA ASO decreases the expression level of an endogenous *SNCA* gene. The neuronal cell can be, but is not limited to, a brain cell, a deep brain cell, or a spinal cord cell. In some embodiments, provide herein is a method of generating an oligodendrocyte with decreased alpha-synuclein expression, the method comprising delivering to the oligodendrocyte a SNCA ASO as described herein or a conjugate comprising a SNCA ASO as described herein, wherein the SNCA ASO decreases the expression level of an endogenous *SNCA* gene.

[0016] Certain embodiments provide a method of modifying a neuronal cell to decrease alpha-synuclein expression, the method comprising delivering to the neuron cell a SNCA ASO as described herein or a conjugate comprising a SNCA ASO as described herein, wherein the SNCA ASO decreases the expression level of an endogenous *SNCA* gene. The neuronal cell can be, but is not limited to, a brain cell, a deep brain cell, or a spinal cord cell. In one embodiment, provide herein is a method of modifying an oligodendrocyte to decrease alpha-synuclein expression, the method comprising delivering to the neuron cell a SNCA ASO as described herein or a conjugate comprising a SNCA ASO as described herein, wherein the SNCA ASO decreases the expression level of an endogenous *SNCA* gene.

[0017] Certain embodiments provide a method of modifying a neuronal cell to decrease alpha-synuclein expression, the method comprising delivering to the neuron cell a SNCA ASO as described herein or a conjugate comprising a SNCA ASO as described herein, wherein the SNCA ASO specifically reduces the expression level of a *SNCA* transcript in the cell. The neuronal cell can be, but is not limited to, a brain cell, a deep brain cell, or a spinal cord cell. In one embodiment, provide herein is a method of modifying an oligodendrocyte to decrease alpha-synuclein expression, the method comprising delivering to the neuron cell a SNCA ASO as described herein or a conjugate comprising a SNCA ASO as described herein, wherein the SNCA ASO specifically reduces the expression level of a *SNCA* transcript in the cell.

[0018] Certain embodiments provide a method of delivering a SNCA ASO to the central nervous system (CNS) of a human subject in need thereof, comprising administering to the subject a composition of as described herein (*e.g.*, a SNCA ASO or SNCA ASO conjugated to

a targeting ligand), wherein said SNCA ASO decreases the expression level of an endogenous *SNCA* gene.

[0019] Delivery of a described SNCA ASO to a neuronal cell or an oligodendrocyte can be used to treat a neurodegenerative disorder. Delivery of a described SNCA ASO to a neuronal cell can be used to treat an alpha-synuclein-associated neurodegenerative disorder. The neurodegenerative disorder can be, but is not limited to, Parkinson's disease. Delivery of a described SNCA ASO to an oligodendrocyte can be used to treat an alpha-synuclein-associated neurodegenerative disorder (*i.e.*, a synucleinopathy). The neurodegenerative disorder can be, but is not limited to, multiple systems atrophy, Lewy body dementia, pure autonomic failure (PAF), and REM sleep behavior disorder (RBD).

[0020] Certain embodiments provide a method of delivering a SNCA ASO to cells of the CNS of a human subject, comprising administering to the subject a composition as described herein (*e.g.*, a SNCA ASO or SNCA ASO conjugated to a targeting ligand), wherein said composition is administered by intrathecal injection.

[0021] Certain embodiments provide a method of delivering a SNCA ASO to cells of the CNS of a human subject, comprising administering to the subject a SNCA ASO as described herein, wherein said SNCA ASO is conjugated to a molecule or delivery vehicle that facilitates transport of the SNCA ASO across the blood brain barrier. The SNCA ASO can be linked to any molecule or delivery vehicle known in the art that facilitates transport across the blood brain barrier. Such molecules and delivery vehicles include, but are not limited to, brain shuttles (*e.g.*, as disclosed in WO2018210898, WO2015101588, WO2023056388, and WO2014033074, each of which is incorporated herein by reference). The cells of the CNS can be, but are not limited to, brain cells, deep brain cells, spinal cord cells, neuronal cells, and oligodendrocytes.

[0022] Certain embodiments provide a method of treating an alpha-synuclein associated neurodegenerative disorder (*i.e.*, a synucleinopathy) in a human subject in need thereof, the method comprising administering to the human subject a composition as described herein (*e.g.*, a SNCA ASO or SNCA ASO conjugated to a targeting ligand).

[0023] Certain embodiments provide a method of treating Parkinson's disease, the method comprising administering to a human subject in need thereof, a composition (*e.g.*, a SNCA ASO or SNCA ASO conjugated to a targeting ligand) as described herein.

[0024] Certain embodiments provide a method of reducing *SNCA* messenger ribonucleic acid (mRNA) expression in a human subject in need thereof, the method comprising

administering to the human subject a composition (*e.g.*, a SNCA ASO or SNCA ASO conjugated to a targeting ligand) as described herein.

[0025] Certain embodiments provide a composition as described herein (*e.g.*, a SNCA ASO or SNCA ASO conjugated to a targeting ligand) for use in treating an alpha-synuclein associated neurodegenerative disorder in a human subject in need thereof.

[0026] Certain embodiments provide a composition as described herein (*e.g.*, a SNCA ASO or SNCA ASO conjugated to a targeting ligand) for use in treating Parkinson's disease in a human subject in need thereof.

[0027] Certain embodiments provide a composition as described herein (*e.g.*, a SNCA ASO or SNCA ASO conjugated to a targeting ligand) for use in reducing *SNCA* mRNA expression in a human subject in need thereof.

[0028] Certain embodiments provide the use of a SNCA ASO in the preparation of a medicament for reducing *SNCA* mRNA expression in a human subject in need thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0029] **FIG. 1** illustrates (panel A) SNCA knockdown in brain with 25 µg or 50 µg ICV injection, (panel B) SNCA knockdown in spinal cord with 25 µg or 50 µg ICV injection, and (panel C) glial activation in brain.

[0030] **FIG. 2** illustrates ED50 curves (A) in brain and (B) in spinal cord.

[0031] **FIG. 3** illustrates total ASO concentrations in brain after 25µg or 50 µg ICV injection.

DETAILED DESCRIPTION

[0032] Antisense oligonucleotides are single-stranded small synthetic nucleic acid polymers that can be used to modulate gene expression. They can target pre-mRNA, mRNA, or non-coding RNA to induce degradation, modulate splicing events, or interfere with protein translation. Described herein are antisense oligonucleotides that target *SNCA* RNA transcripts (*e.g.*, a *SNCA* mRNA, such as a pre-mRNA or mature mRNA) and reduce the expression level of the *SNCA* gene.

[0033] The *SNCA* gene encodes alpha-synuclein protein (also referred herein as aSyn or SNCA), a presynaptic neuronal protein. Alpha-synuclein protein is predominantly expressed in neurons and is enriched in presynaptic terminals. Structurally, it is characterized by three distinct regions: an amphipathic N-terminal domain, a central hydrophobic region known as the non-amyloid beta component (NAC), and an acidic C-terminal domain. The N-terminal

domain is implicated in membrane binding, while the NAC region is crucial for alpha-synuclein aggregation. Functionally, alpha-synuclein participates in the regulation of synaptic vesicle dynamics and neurotransmitter release. However, under pathological conditions, alpha-synuclein undergoes misfolding, leading to the formation of oligomers and insoluble fibrils, which contribute to the development of neurodegenerative diseases. SNCA is associated with several neurodegenerative disorders, such as Parkinson's disease, dementia with Lewy bodies (DLB), multiple system atrophy (MSA), PAF, and RBD. In one embodiment, a human *SNCA* gene has the sequence identified in GENBANK Accession No. NG_011851.1. In one embodiment, a *SNCA* pre-mRNA has the sequence of SEQ ID NO: 48 (Ensembl ENST00000394991). An embodiment of a mRNA transcript is provided as SEQ ID NO: 49 (refseq NM_000345).

[0034] The SNCA ASOs described herein can be used to treat Asyn-associated disorders, *e.g.*, Parkinson's disease.

[0035] As used herein, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise.

[0036] As used herein, the terms "about" and "approximately," when used to modify an amount specified in a numeric value or range, indicate that the numeric value as well as reasonable deviations from the value known to the skilled person in the art, for example $\pm 20\%$, $\pm 10\%$, or $\pm 5\%$, are within the intended meaning of the recited value.

[0037] The term "antisense oligonucleotide (ASO)" refers to single strands of DNA-like or RNA-like molecules (*e.g.*, comprising a modified internucleoside linkage(s), a modified nucleobase(s), and/or a modified sugar(s), such as those described herein) that are complementary or partially complementary to a chosen target polynucleotide sequence, *e.g.*, an mRNA. By binding to a complementary target sequence ASOs can alter or modulate gene expression through a number of mechanisms, including, *e.g.*, by altering splicing (exon exclusion or exon inclusion); by recruiting RNase H leading to target degradation; through translation inhibition; and by small RNA inhibition.

[0038] As used herein, a "SNCA antisense oligonucleotide" or "SNCA ASO" or "SNCA targeting antisense oligonucleotide" refers to an ASO that is capable of binding (hybridizing) to a *SNCA* target nucleic acid (*e.g.*, an RNA transcript, such a *SNCA* mRNA (*e.g.*, a *SNCA* pre-mRNA or a *SNCA* mature mRNA) or cDNA) in a sequence specific manner, resulting the reduction of *SNCA* gene expression.

[0039] A "transferrin receptor" or "TfR" as used herein refers to transferrin receptor protein 1. Transferrin receptor protein 1 sequences from other species are also known (*e.g.*,

chimpanzee, accession number XP_003310238.1; rhesus monkey, NP_001244232.1; dog, NP_001003111.1; cattle, NP_001193506.1; mouse, NP_035768.1; rat, NP_073203.1; and chicken, NP_990587.1). The term “transferrin receptor” also encompasses allelic variants of exemplary reference sequences, *e.g.*, human sequences, that are encoded by a gene at a transferrin receptor protein 1 chromosomal locus. Full length transferrin receptor protein includes a short N-terminal intracellular region, a transmembrane region, and a large extracellular domain. The extracellular domain is characterized by three domains: a protease-like domain, a helical domain, and an apical domain.

[0040] The terms “identical” or percent “identity,” in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues, *e.g.*, at least 60% identity, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% or greater, that are identical over a specified region when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one a sequence comparison algorithm or by manual alignment and visual inspection.

[0041] For sequence comparison of polypeptides, typically one amino acid sequence acts as a reference sequence, to which a candidate sequence is compared. Alignment can be performed using various methods available to one of skill in the art, *e.g.*, visual alignment or using publicly available software using known algorithms to achieve maximal alignment. Such programs include the BLAST programs, ALIGN, ALIGN-2 (Genentech, South San Francisco, Calif.) or Megalign (DNASTAR). The parameters employed for an alignment to achieve maximal alignment can be determined by one of skill in the art. For sequence comparison of polypeptide sequences for purposes of this application, the BLASTP algorithm standard protein BLAST for aligning two proteins sequence with the default parameters is used.

[0042] The terms “corresponding to,” “determined with reference to,” or “numbered with reference to” when used in the context of the identification of a given nucleotide residue in a nucleic acid sequence, refers to the position of the residue of a specified reference sequence when the given nucleotide sequence is maximally aligned and compared to the reference sequence. The nucleic acid sequence that is aligned to the reference sequence need not be the same length as the reference sequence.

[0043] As used herein, the term “nucleic acid” and “polynucleotide” refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, composed of monomers (nucleotides) containing a sugar moiety, phosphate and

a nucleobase. Unless specifically limited, the term encompasses both modified and unmodified nucleic acids.

[0044] As used herein, the term “nucleobase” refers to nitrogen-containing compounds that can be linked to a sugar moiety to form nucleosides, which in turn are components of nucleotides. The ability of nucleobases to form base pairs and to stack one upon another leads directly to long-chain helical structures such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Nucleobases may be naturally occurring (*i.e.*, adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U)) or modified (*e.g.*, 5-methyl cytosine). Thus, the nucleobase moieties may be shown by the corresponding letter code for each nucleobase, *e.g.*, A, T, G, C or U, wherein each letter may optionally include modified nucleobases that are functionally equivalent (*e.g.*, based on Watson Crick base pairing ability).

[0045] As used herein, the term “nucleoside” refers to a compound comprising a nucleobase and sugar moiety (*e.g.*, deoxyribose or ribose, or a modified variant thereof). The term nucleoside includes both modified and unmodified nucleosides.

[0046] As used herein, the term “nucleotide” refers to a compound comprising a nucleobase, a sugar moiety, and one or more phosphate groups. The term nucleotide includes both modified and unmodified nucleotides.

[0047] As used herein, the term “internucleoside linkage” means the covalent linkages between two nucleosides in an oligonucleotide. Nucleosides may be linked via natural (*i.e.*, a phosphodiester (PO) linkage) or modified linkages.

[0048] The terms “chemical modification”, “modification” or “modified” may refer to a chemical change in a compound when compared to its naturally occurring counterpart. For example, a nucleobase, a sugar moiety or an internucleoside linkage may be chemically modified.

[0049] The terms “nucleotide sequence” and “nucleic acid sequence” and “nucleic acid strand” refer to a sequence of bases (purines and/or pyrimidines, or synthetic derivatives thereof) in a polymer of DNA or RNA, which can be single-stranded or double-stranded, optionally containing synthetic, non-natural or altered nucleotides capable of incorporation into DNA or RNA polymers, and/or backbone modifications (*e.g.*, a modified oligomer). The terms “oligo”, “oligonucleotide” and “oligomer” may be used interchangeably and refer to such sequences of purines and/or pyrimidines. For example, the oligonucleotide may comprise chemically modified or unmodified nucleic acid molecules (RNA or DNA) having a length of less than about, *e.g.*, about 200 nucleotides (for example, less than about 100 or 50 nucleotides). The oligonucleotide can, *e.g.*, be single stranded DNA or RNA (*e.g.*, an ASO); double stranded

DNA or RNA (*e.g.*, small interfering RNA (siRNA)), including double stranded DNA or RNA having a hairpin loop; or DNA/RNA hybrids. In one embodiment, the oligonucleotide has a length ranging from about 5 to about 60 nucleotides, or about 10 to about 50 nucleotides. In another embodiment, the oligonucleotide has a length ranging from about 5 to about 30 nucleotides or from about 12 to about 30 nucleotides. In yet another embodiment, the oligonucleotide has a length ranging from about 10 to about 25 nucleotides. In yet another embodiment, the oligonucleotide has a length ranging from about 16 to about 20 nucleotides.

[0050] The terms “modified oligos”, “modified oligonucleotides” or “modified oligomers” may be similarly used interchangeably, and refer to such sequences that contain synthetic, non-natural or altered bases, sugars and/or backbone modifications.

[0051] A “modified nucleotide” is a nucleotide other than a ribonucleotide (2'-hydroxyl nucleotide) or a deoxyribonucleotide (2'-H nucleotide). A modified nucleotide can comprise one or more of: a modified nucleobase, a modified ribose (sugar) moiety, and a modified internucleoside linkage to another nucleoside. Modified nucleotides include nucleotide mimics. Modified nucleosides include abasic nucleosides, which lack a nucleobase, and nucleosides in which the ribose is substituted for a non-sugar moiety (*e.g.*, a sugar surrogate, *e.g.*, a morpholino or as in a peptide nucleic acid).

[0052] A “2'-deoxynucleoside” is a nucleoside comprising a 2'-deoxyribose sugar moiety. In naturally occurring DNA, 2'-deoxynucleosides comprise a ribose having a β -D ribosyl configuration.

[0053] A “2'-substitution modified nucleoside” or “2' substituted nucleoside” or “2' modified nucleoside” is a nucleoside comprising a 2'-substitution (*e.g.*, a group other than hydrogen or hydroxyl) at the 2'-OH group of a ribosyl sugar moiety. A 2' substituted nucleoside comprises at least one 2'-substituent group other than H or OH at the 2' carbon of the nucleoside ribose.

[0054] “2'-MOE modified nucleoside” or “2'-MOE nucleoside” is a nucleoside comprising a 2'-OCH₂CH₂OCH₃ (O-methoxyethyl) substitution at the 2'-OH group of a ribosyl sugar moiety.

[0055] A “2'-NMA modified nucleoside” or “2'-NMA nucleoside” is a nucleoside comprising 2'-O-CH₂-C(=O)-NH-CH₃ (O-N-methyl acetamide) substitution at the 2'-OH group of a ribosyl sugar moiety.

[0056] A “2'-OMe modified nucleoside” or “2'-OMe nucleoside” is a nucleoside comprising a 2'-OCH₃ substitution at the 2'-OH group of a ribosyl sugar moiety.

[0057] A “2'-F modified nucleoside” or “2'-F nucleoside” is a nucleoside comprising a 2'-fluoro substitution in place of the 2'-OH group of a ribosyl sugar moiety.

[0058] A “bicyclic nucleoside” (also termed bridged nucleoside) is a nucleoside comprising a bicyclic sugar moiety. A “bicyclic sugar” or “bicyclic sugar moiety” is a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In some embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety, such as a ribosyl sugar moiety of a nucleoside.

[0059] A “non-bicyclic modified sugar moiety” is a modified sugar moiety that comprises a modification, such as a substituent, that does not form a bridge between two atoms of the sugar to form a second ring.

[0060] A “constrained ethyl” or “cEt” or “cEt modified sugar moiety” or “cEt sugar moiety” is a β -D ribosyl bicyclic sugar moiety wherein the second ring of the bicyclic sugar is formed via a bridge connecting the 4'-carbon and the 2'-carbon of the β -D ribosyl sugar moiety, wherein the bridge has the formula 4'-CH(CH₃)-O-2', and wherein the methyl group of the bridge is in the S configuration. A “cEt modified nucleoside” or “cEt nucleoside” is a nucleoside comprising a cEt modified sugar moiety.

[0061] A “locked nucleic acid” or “LNA nucleoside” or “LNA” is a bicyclic nucleoside having a 4'-CH₂-O-2' bridge between the 4' and the 2' furanosyl ring atoms.

[0062] A “sugar surrogate” is a moiety having other than a ribosyl moiety of a modified nucleoside. Oligonucleotides comprising one or more suitable sugar surrogates retain the ability to hybridize to complementary target nucleobase or nucleic acid sequences.

[0063] An “internucleoside linkage” is the covalent linkage between adjacent nucleosides in an oligonucleotide. As used herein “modified internucleoside linkage” means any internucleoside linkage other than a phosphodiester internucleoside linkage.

[0064] A “phosphorothioate internucleoside linkage” is a modified internucleoside linkage in which one of the non-bridging oxygen atoms of a phosphodiester internucleoside linkage is replaced with a sulfur atom.

[0065] A “5-methylcytosine” comprises a cytosine modified with a methyl group attached to the 5 position. A 5-methylcytosine is a modified nucleobase.

[0066] An “abasic nucleoside” comprises a nucleoside lacking a nucleobase.

[0067] A “chirally enriched population” means a plurality of molecules of identical molecular formula, wherein the number or percentage of molecules within the population that contain a particular stereochemical configuration at a particular chiral center is greater than the

number or percentage of molecules expected to contain the same particular stereochemical configuration at the same particular chiral center within the population if the particular chiral center were stereorandom. Chirally enriched populations of molecules having multiple chiral centers within each molecule may contain one or more stereorandom chiral centers. In some embodiments, the molecules are modified oligonucleotides.

[0068] A “stabilized phosphate group” refers to a 5'-chemical moiety that results in stabilization of a 5'-phosphate moiety of the 5'-terminal nucleoside of an oligonucleotide, relative to the stability of an unmodified 5'-phosphate of an unmodified nucleoside under biologic conditions. Stabilized phosphate groups include, but are not limited to, 5'-vinyl phosphonates and 5'-cyclopropyl phosphonate.

[0069] The SNCA ASOs described herein may be synthesized using standard solid or solution phase synthesis techniques that are known in the art. In certain embodiments, the SNCA ASOs are synthesized using solid-phase phosphoramidite chemistry (U.S. Patent No. 6,773,885) with automated synthesizers. Chemical synthesis of nucleic acids allows for the production of various forms of the nucleic acids with modified linkages, chimeric compositions, and nonstandard bases or modifying groups attached in chosen places through the nucleic acid's entire length.

[0070] The term “complementary” as used herein refers to the broad concept of complementary base pairing between two nucleic acids aligned in an antisense position in relation to each other. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are substantially complementary to each other when at least about 50%, at least about 60%, or at least about 80% of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (*e.g.*, A:T (A:U for RNA) and G:C nucleotide pairs).

[0071] The term percent “complementary,” in the context of two or more nucleotide sequences, refer to two or more sequences or subsequences that are the complementary or have a specified percentage of nucleotides, *e.g.*, at least 60% identity, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% or greater, that are complementary over a specified region when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one a sequence comparison algorithm or by manual alignment and visual inspection.

[0072] The terms “identical” or percent “identity,” in the context of two or more nucleotide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides, *e.g.*, at least 60% identity, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% or greater, that are identical over a specified region when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one a sequence comparison algorithm or by manual alignment and visual inspection.

[0073] It will be understood that in determining percent complementarity or percent identity, chemical modifications are disregarded if the functional capacity of the nucleobase to form Watson Crick base pairing is retained (*e.g.*, 5-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

[0074] For sequence comparison of oligonucleotides (*e.g.*, to determine identity or complementarity), typically one nucleotide sequence acts as a reference sequence, to which a candidate sequence is compared. Alignment can be performed using various methods available to one of skill in the art, *e.g.*, visual alignment or using publicly available software using known algorithms to achieve maximal alignment. Such programs include the BLAST programs, ALIGN, ALIGN-2 (Genentech, South San Francisco, Calif.) or Megalign (DNASTAR). The parameters employed for an alignment to achieve maximal alignment can be determined by one of skill in the art.

[0075] As used herein, “hybridize” or “hybridization” means the pairing of complementary nucleotide sequences (*e.g.*, an antisense compound and its target nucleic acid; or between antisense and sense strands). As used herein, “specifically hybridizes” means the ability of a reference nucleic acid to hybridize to one nucleic acid molecule with greater affinity than it hybridizes to another.

[0076] “Expression” refers to the transcription and/or translation of an endogenous gene, heterologous gene or nucleic acid segment, or a transgene in cells. For example, expression may refer to the transcription and stable accumulation of sense (mRNA) or functional RNA. Expression may also refer to the production of protein.

[0077] The term “gene” refers to a nucleic acid (*e.g.*, DNA or RNA) sequence that comprises coding sequences necessary for the production of a polypeptide or precursor.

[0078] The phrase “modulating the expression of a target gene or sequence” means a change (*e.g.*, an increase or decrease) in expression of the target gene or sequence (*e.g.*, via degradation of the target or translation inhibition). For example, it includes inhibiting, reducing or decreasing the expression of a target gene or sequence. This also includes a change in

alternative splicing, which may result in a change in the absolute or relative amount of a particular splice variant.

[0079] The term “subject,” “individual,” and “patient,” as used interchangeably herein, refer to a mammal, including but not limited to humans, non-human primates, rodents (*e.g.*, rats, mice, and guinea pigs), rabbits, cows, pigs, horses, and other mammalian species. In one embodiment, the patient is a human.

[0080] The terms “treatment,” “treating,” and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. “Treating” or “treatment” may refer to any indicia of success in the treatment or amelioration of an injury, disease, or condition, including any objective or subjective parameter such as abatement, remission, improvement in patient survival, increase in survival time or rate, diminishing of symptoms or making the injury, disease, or condition more tolerable to the patient, slowing in the rate of degeneration or decline, or improving a patient’s physical or mental well-being. Additionally, “treating” or “treatment” may refer to the modulation of the target gene expression such as gene knockdown or gene knockout. For instance, the expression of the target gene or sequence is inhibited or reduced, *e.g.*, by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or 100%, as compared to the expression in a control. The treatment or amelioration of symptoms can be based on objective or subjective parameters. The effect of treatment can be compared to an individual or pool of individuals not receiving the treatment, or to the same patient prior to treatment or at a different time during treatment.

[0081] As used herein, “ameliorate” refer to the use of an effective amount of a SNCA ASO and to conjugates comprising the same for improving at least one symptom (*e.g.*, as compared to the same symptom in the absence of the treatment). In certain embodiments, amelioration is the reduction in the severity or frequency of a symptom or the delayed onset or slowing of progression in the severity or frequency of a symptom.

[0082] The terms “prevent” or “prevention” refer to the use of an effective amount of a SNCA ASO and to conjugates comprising the same for reducing or eliminating the occurrence or recurrence of a disease or a disease symptom in a subject, such as in a subject prone to developing or re-developing the disease.

[0083] The term “pharmaceutically acceptable excipient” refers to a non-active pharmaceutical ingredient that is biologically or pharmacologically compatible for use in humans or animals, such as but not limited to a buffer, carrier, or preservative.

[0084] As used herein, a “therapeutic amount” or “therapeutically effective amount” of an agent is an amount of the agent that treats, alleviates, abates, or reduces the severity of

symptoms of a disease in a subject. A “therapeutic amount” or “therapeutically effective amount” of an agent may improve patient survival, increase survival time or rate, diminish symptoms, make an injury, disease, or condition more tolerable, slow the rate of degeneration or decline, or improve a patient’s physical or mental well-being.

[0085] The term “administer” refers to a method of delivering agents, compounds, or compositions to the desired site of biological action. These methods include, but are not limited to, topical delivery, parenteral delivery, intravenous delivery, intradermal delivery, intramuscular delivery, intrathecal delivery, colonic delivery, rectal delivery, or intraperitoneal delivery. In one embodiment, the proteins described herein are administered intravenously.

[0086] The term “control” or “control value” refers to a reference value or baseline value. Appropriate controls can be determined by one skilled in the art. In some instances, control values can be determined relative to a baseline within the same subject or experiment, *e.g.*, a measurement of *SNCA* gene expression taken prior to treatment with a *SNCA* ASO as described herein or a conjugate or composition thereof can be a control value for a post-treatment measurement of *SNCA* levels in the same subject. In other instances, the control value can be determined relative to a control subject (*e.g.*, a healthy control or a disease control) or an average value in a population of control subjects (*e.g.*, healthy controls or disease controls, *e.g.*, a population of 10, 20, 50, 100, 200, 500, 1000 control subjects or more), *e.g.*, a measurement of a subject’s level of *SNCA* gene expression either at baseline or after treatment can be compared to a healthy control value.

3. *SNCA* TARGETING OLIGONUCLEOTIDES

[0087] Disclosed herein are *SNCA* antisense oligonucleotides (*SNCA* ASOs) complementary to the human *SNCA* gene. The *SNCA* ASOs as described herein are at least 90% complementary to the human *SNCA* gene. In some embodiments, the *SNCA* ASOs as described herein are at least 90% complementary to SEQ ID NO: 49. In some embodiments, a *SNCA* ASO as described herein comprises or consists of a nucleic acid sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to SEQ ID NO: 49. In some embodiments, a *SNCA* ASO as described herein comprises or consists of a nucleic acid sequence 10 to 25 nucleotides in length that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to SEQ ID NO: 49. In some embodiments, a *SNCA* ASO as described herein comprises or consists of a nucleic acid sequence 10 to 25 nucleotides in length

that is at least 90% or at least 95% complementary to SEQ ID NO: 49. In some embodiments, a SNCA ASO as described herein comprises or consists of a nucleic acid sequence 10 to 25 nucleotides in length that is 100% complementary to SEQ ID NO: 49.

[0088] ASOs range from about 10 to 30 base pairs (bp) in length, but may be longer or shorter. For example, in certain embodiments, the ASO is about 10 to about 60 nucleotides in length (*i.e.*, about 10 to about 60 linked nucleosides in length), or about 10 to about 50 nucleotides in length, or about 10 to about 40 nucleotides in length. In certain embodiments, the ASO is about 10 to 30 nucleotides in length, or about 12 to 30 nucleotides in length, or about 14 to about 30 nucleotides in length, or about 15 to about 30 nucleotides in length, or about 16 to about 30 nucleotides in length, or about 17 to about 30 nucleotides in length, or about 18 to about 30 nucleotides in length, or about 18 to about 28 nucleotides in length or about 18 to 26 nucleotides in length, or about 18 to about 24 nucleotides in length, or about 15 to about 25 nucleotides in length, or about 16 to about 20 nucleotides in length. In certain embodiments, the ASO is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length.

[0089] While the length of the SNCA ASO may vary, in certain embodiments, the SNCA ASO is from about 10 to about 60 nucleotides in length, or from about 10 to about 30 nucleotides in length, or from about 18 to about 30 nucleotides in length or from about 15 to about 25 nucleotides in length, or from about 16 to about 20 nucleotides in length. In some embodiments, the ASO is about 16 to about 20 nucleotides in length. In some embodiments, the ASO is 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, the ASO is 16 nucleotides in length. In some embodiments, the ASO is 17 nucleotides in length. In some embodiments, the ASO is 18 nucleotides in length. In some embodiments, the ASO is 19 nucleotides in length. In some embodiments, the ASO is 20 nucleotides in length.

[0090] In some embodiments, a SNCA ASO disclosed herein comprises or consists of a nucleic acid sequence comprising or consisting of at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleobases of a sequence selected from any one of SEQ ID NOs: 1-15 and 18-23 and at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, a SNCA ASO disclosed herein comprises a nucleic acid sequence selected from any one of SEQ ID NOs: 1-15 and 18-23 and further comprises at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, a SNCA ASO disclosed herein consists of a nucleic acid sequence selected from any one of SEQ ID NOs: 1-15 and 18-23 and further comprises at least one modified internucleoside linkage and/or at least one

modified sugar. In some embodiments, the SNCA ASO disclosed herein targets an exon of the *SNCA* gene and comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 1 and 6-8 and further comprises at least one modified internucleoside linkage and/or at least one modified sugar. In one embodiment, the SNCA ASO disclosed herein targets an intron of the *SNCA* gene and comprises or consists of a nucleic acid sequence selected from any one of SEQ ID NOs: 2 and 10-12 and further comprises at least one modified internucleoside linkage and/or at least one modified sugar. In one embodiment, the SNCA ASO disclosed herein targets an intron of the *SNCA* gene and comprises or consists of a nucleic acid sequence selected from any one of SEQ ID NOs: 3 and 13-15 and further comprises at least one modified internucleoside linkage and/or at least one modified sugar. In one embodiment, the SNCA ASO disclosed herein targets an intron of the *SNCA* gene and comprises or consists of a nucleic acid sequence selected from any one of SEQ ID NOs: 4 and 18-20 and further comprises at least one modified internucleoside linkage and/or at least one modified sugar. In one embodiment, the SNCA ASO disclosed herein targets an intron of the *SNCA* gene and comprises or consists of a nucleic acid sequence selected from any one of SEQ ID NOs: 5 and 21-23 and further comprises at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, the SNCA ASOs disclosed herein comprise 15 to 25 linked nucleosides. In some embodiments, the SNCA ASOs disclosed herein comprise 16 to 20 linked nucleosides. In some embodiments, the SNCA ASOs disclosed herein comprise 17 to 19 linked nucleosides. In some embodiments, the SNCA ASO disclosed herein (*e.g.*, 15 to 25 or 17 to 19 nucleotides in length) are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to SEQ ID NO: 1.

[0091] In some embodiments, a SNCA ASO disclosed herein is 16-20 nucleotides in length, and comprises the sequence of any one of SEQ ID NOs: 24-38 and 41-46, wherein the SNCA ASO further comprises at least one modified internucleoside linkage and/or at least one modified sugar. In some embodiments, a SNCA ASO disclosed herein comprises a gapmer ASO having a gap segment comprising the sequence of any one of SEQ ID NOs: 24-38 and 41-46. In some embodiments, a SNCA ASO disclosed herein comprises a gapmer ASO having a gap segment consisting of the sequence of any one of SEQ ID NOs: 24-38 and 41-46. In some embodiments, a SNCA ASO comprises a sequence comprising 100% identity to any of the SEQ ID NOs: 24-38 and 41-46 and at least 85%, at least 90%, at least 95% or 100% identity to any of SEQ ID NOs: 1-15 and 18-23.

Table 1. SNCA gapmer sequences

SEQ ID NO	Sequence
1	CACATTGGAAGTGAAGT
2	GTTAAATCTAGTTGTCCA
3	TCTCTATATAACATCACT
4	AACTGCTTAGTGATTCCA
5	GGTAACTTAGGACAAGGT

Table 2. SNCA 16mer gapmer sequences

SEQ ID NO	Sequence
6	CACATTGGAAGTGAAGC
7	ACATTGGAAGTGAAGCA
8	CATTGGAAGTGAAGCAC
9	ATTGGAAGTGAAGCACT
10	GTTAAATCTAGTTGTCT
11	TTAAATCTAGTTGTCC
12	TAAATCTAGTTGTCCA
13	TCTCTATATAACATCA
14	CTCTATATAACATCAC
15	TCTATATAACATCACT
18	AACTGCTTAGTGATTCT
19	ACTGCTTAGTGATTCC
20	CTGCTTAGTGATTCCA
21	GGTAACTTAGGACAAG
22	GTAACCTTAGGACAAGG
23	TAACTTAGGACAAGGT

Table 3. SNCA Gap sequences

SEQ ID NO	Sequence
24	ATTGGAAGTGAAGC
25	AAATCTAGTTGT
26	CTATATAACATC
27	TGCTTAGTGATT
28	AACTTAGGACAA

Table 4. SNCA 10mer Gap sequences

SEQ ID NO	Sequence
29	ATTGGAAGTG
30	TTGGAAGTGA
31	TGGAAGTGAG
32	GGAAGTGAGC
33	AAATCTAGTT
34	AATCTAGTTG
35	ATCTAGTTGT
36	CTATATAACA
37	TATATAACAT
38	ATATAACATC
41	TGCTTAGTGA
42	GCTTAGTGAT
43	CTTAGTGATT
44	AACTTAGGAC
45	ACTTAGGACA
46	CTTAGGACAA

Illustrative Oligonucleotide Modifications

[0092] In certain embodiments, a SNCA ASO described herein may comprise at least one nucleic acid modification, such as those selected from the group consisting of a modified internucleoside linkage, a modified nucleobase, a modified sugar, and combinations thereof (*e.g.*, comprises at least one modified internucleoside linkage and/or at least one modified sugar). Such modifications may be used to alter pharmacokinetics (improved nuclease resistance resulting in a longer half-life), pharmacodynamics (superior affinity for the target RNA), or endocytic uptake. However, many modifications preclude cleavage by RNase H, which is the desired mechanism of action for many ASOs. Thus, certain RNase H ASOs may be designed as chimeras, where different bases are a mix of different chemistries, or as gapmers, where some modifications are placed on the “wings” and not the central bases.

[0093] Accordingly, a SNCA ASO described herein may comprise one or more nucleic acid modifications. In certain embodiments, an SNCA ASO comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 40, or more modifications. In certain embodiments, a SNCA ASO described herein comprises one or more nucleotide modifications (*e.g.*, to the nucleobase or sugar moiety). In certain embodiments,

25% or more of the nucleotides present in the SNCA ASO are modified. In certain embodiments, 50% or more of the nucleotides present in the SNCA ASO are modified. In certain embodiments, 75% or more of the nucleotides present in the SNCA ASO are modified. In certain embodiments, 100% of the nucleotides present in the SNCA ASO are modified.

Modified sugar moiety, nucleosides/nucleotides

[0094] In certain embodiments, the SNCA ASO comprises one or more nucleobase modifications. In certain embodiments, the SNCA ASO comprises one or more modifications to the sugar moiety (*e.g.*, furanosyls comprising substitutions at the 2'-position, the 3'-position, the 4'-position and/or the 5'-position). In certain embodiments, substituted sugar moieties include bicyclic sugar moieties. In some embodiments, the bicyclic sugar moieties comprise a chemical bridge between the 4' and 2' positions of the sugar, wherein each chemical bridge is independently selected from: 4'-CH(R)-O-2' and 4'-(CH₂)₂-O-2', wherein each R is independently selected from H, C₁-C₆ alkyl and C₁-C₆ alkoxy. In one embodiment, the bicyclic sugar moieties comprise a chemical bridge between the 4' and 2' positions of the sugar, wherein each chemical bridge is 4'-CH(R)-O-2' and wherein each R is independently H.

[0095] Modified nucleosides/nucleotides include, but are not limited to, 2'-O methyl (2'OMe) residues, 2' O-methoxyethyl (MOE) residues, constrained nucleic acid residues (*e.g.*, S-cEt, R-cEt, S-cMOE, and R-cMOE), peptide nucleic acid (PNA) residues, locked nucleic acid (LNA) residues, and 5-methylcytidine residues (methylated cytosine residues) (*see, also*, Scoles, et al., *Neurol Genet* Apr 2019, 5 (2) e323). In certain embodiments, the SNCA ASO comprises one or more 2'-MOE residues. In certain embodiments, the SNCA ASO comprises one or more OMe residues or F residues (*e.g.*, 2'-F or 2'OMe). In certain embodiments, the SNCA ASO comprises one or more constrained (*e.g.*, S-cEt, R-cEt, S-cMOE, and R-cMOE) and/or LNA residues. Nucleic acids are considered "locked" when they have a methylene bridge connection made between 2'-oxygen and the 4'-carbon of the ribose sugar molecule. In certain embodiments, the SNCA ASO is a morpholino (*i.e.*, comprises certain modifications to the sugar moiety).

[0096] In certain embodiments, a SNCA ASO described herein comprises one or more LNA residues. In certain embodiments, a SNCA ASO described herein comprises one or more 5-methylcytidine residues. In certain embodiments, a SNCA ASO described herein comprises one or more LNA residues and one or more 5-methylcytidine residues. In some embodiments, every cytosine in a SNCA ASO is a 5-methylcytidine.

Modified internucleoside linkage

[0097] In certain embodiments, the SNCA ASO comprises one or more modifications to the internucleoside backbone (*i.e.*, the natural phosphodiester (PO) linkage is modified). In certain embodiments, such modifications are made to, *e.g.*, reduce nuclease activity. Thus, in certain embodiments, a SNCA ASO comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more modified internucleoside linkages. In certain embodiments, 25% or more of the internucleoside linkages are modified. In certain embodiments, 50% or more of the internucleoside linkages are modified. In certain embodiments, 75% or more of the internucleoside linkages are modified. In certain embodiments, 100% of the internucleoside linkages present in the SNCA ASO are modified.

[0098] Backbone modifications are known in the art and include, but are not limited, to, phosphorothioate (PS) linkages, chiral phosphorothioate linkages, phosphorodiamidate linkages, phosphorodithioate linkages, aminoalkylphosphotriester linkages, phosphotriester linkages, thiophosphate linkages, phosphonate linkages, methyl phosphonate linkages, alkyl phosphonate linkages, 3' alkylene phosphonate linkages, chiral phosphonate linkages, 3'-amino phosphoramidate linkages, aminoalkylphosphoramidate linkages, phosphinate linkages, thionoalkylphosphonate linkages, thionophosphoramidate linkages, thionoalkyl-phosphotriester linkages, borano-phosphate linkages, morpholino linkages and peptide nucleic acid (PNA) linkages. For example, in certain embodiments, one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more) of the internucleoside linkages in the SNCA ASO are replaced with a phosphorothioate (PS) linkage. In certain embodiments, the SNCA ASO comprises a mix of modified and unmodified linkages. The modification at one internucleoside linkage can be independent of the modification at another internucleoside linkage. In certain embodiments, every internucleoside linkage in a SNCA ASO is a modified linkage. In certain embodiments, every internucleoside linkage in a SNCA ASO is a PS linkage. In some embodiments, every internucleoside linkage in an SNCA ASO is a phosphorothioate. In certain other embodiments, one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) of the internucleoside linkages in the SNCA ASO are replaced with a phosphorodiamidate linkage. In certain embodiments, the SNCA ASO is a phosphorodiamidate morpholino (PMO).

[0099] In certain embodiments, the internucleoside linkages are stereorandom with regard to the chiral centers (Rp and Sp). In certain other embodiments, the Rp and Sp configurations in the SNCA ASO are optimized in particular configurations.

Antisense Oligonucleotide Motifs

[0100] In certain embodiments, the nucleic acid modifications with the SNCA ASO are included in a pattern. In certain embodiments, the SNCA ASO is a gapmer. The modification pattern of a gapmer SNCA ASO generally has the formula 5'-X_a-Y_a-Z_a-3', with X_a and Z_a as flanking regions around a gap region Y_a. In certain embodiments, the Y_a region is a contiguous stretch of nucleotides (*i.e.*, linked nucleosides), *e.g.*, a region of at least 6 DNA nucleotides, which are capable of recruiting an RNase, such as RNase H. In certain embodiments, the Y_a region is at least 8 DNA nucleotides. In certain embodiments, the Y_a region is about 9 to about 15 DNA nucleotides. In certain embodiments, the Y_a region is about 11 to about 13 DNA nucleotides. In certain embodiments, the Y_a region is 10, 11, 12, or 13 DNA nucleotides. In certain embodiments, the gapmer binds to the target nucleic acid, at which point an RNase is recruited and can then cleave the target nucleic acid. In certain embodiments, the Y_a region is flanked both 5' and 3' by regions X_a and Z_a, which comprise modified nucleotides, *e.g.*, one to six modified nucleotides in each of X_a and Z_a. In certain embodiments, the Y_a region is flanked both 5' and 3' by regions X_a and Z_a, wherein X_a and Z_a comprise modified nucleotides having modified sugars. In certain embodiments, each nucleotide in X_a and Z_a comprises a modified nucleotide having a sugar modification. The modified nucleotide can be, but is not limited to, a 2-MOE modified nucleotide, a bicyclic nucleotide, a LNA nucleotide, or a cET modified nucleotide. In certain embodiments, the modified nucleotides are present in the 5' and 3' regions of the SNCA ASO, while certain modified nucleotides and/or modified linkages may or may not be present in the central portion of the molecule. In certain embodiments, the modified nucleotides are present in the 5' and 3' regions of the SNCA ASO and certain modified nucleotides are not present in the central portion of the molecule (*e.g.*, LNA residues are not present in the central portion; however, the central region may contain modified linkages, such as PS linkages). In certain embodiments, X_a and Z_a are each independently about 3 to about 6 nucleotides in length. In certain embodiments, X_a and Z_a are each independently 3, 4, or 5 nucleotides in length. In certain embodiments, X_a and Z_a each comprise 3 modified nucleotides (*e.g.*, comprising a modified sugar). In certain embodiments, the 3 modified nucleotides are arranged in tandem in each of X_a and Z_a.

[0101] In certain embodiments, the SNCA ASO is a gapmer comprising LNA and PS modifications. For example, in certain embodiments, the SNCA ASO is a gapmer having a modification pattern of the formula 5'-X_a-Y_a-Z_a-3', with X_a and Z_a as flanking regions around a gap region Y_a, wherein X_a and Z_a each comprise 3 LNA modified nucleotides (*e.g.*, 3 consecutive LNA modified nucleotides), and wherein the gap region Y_a comprises PS linkages.

In some embodiments, every internucleotide linkage in the SNCA ASO comprises a PS linkage. In some embodiments, the SNCA ASO contains a mixture of PS linkages and another modified internucleoside linkage. In certain embodiments, the SNCA ASO further comprises one or more 5-methylcytidine residues. In certain embodiments, the gap region Y_a does not comprise LNA residues.

[0102] In certain embodiments, the SNCA ASO comprises from 5' to 3': a 5' wing segment having from 1 to 6 nucleosides, wherein each nucleoside of the 5' wing segment comprises a modified sugar; a gap segment having from 8 to 15 nucleosides, wherein each nucleoside of the gap segment is a deoxynucleoside; and a 3' wing segment having from 1 to 6 nucleosides, wherein each nucleoside of the 3' wing segment comprises a modified sugar. In some embodiments, every internucleotide linkage in the SNCA ASO comprises a PS linkage. In some embodiments, the SNCA ASO contains a mixture of PS linkages and another modified internucleoside linkage. In some embodiments, every nucleoside in 5' and 3' wing segments comprises a 2-MOE modified nucleotide, a bicyclic nucleotide, a LNA nucleotide, or a cET modified nucleotide.

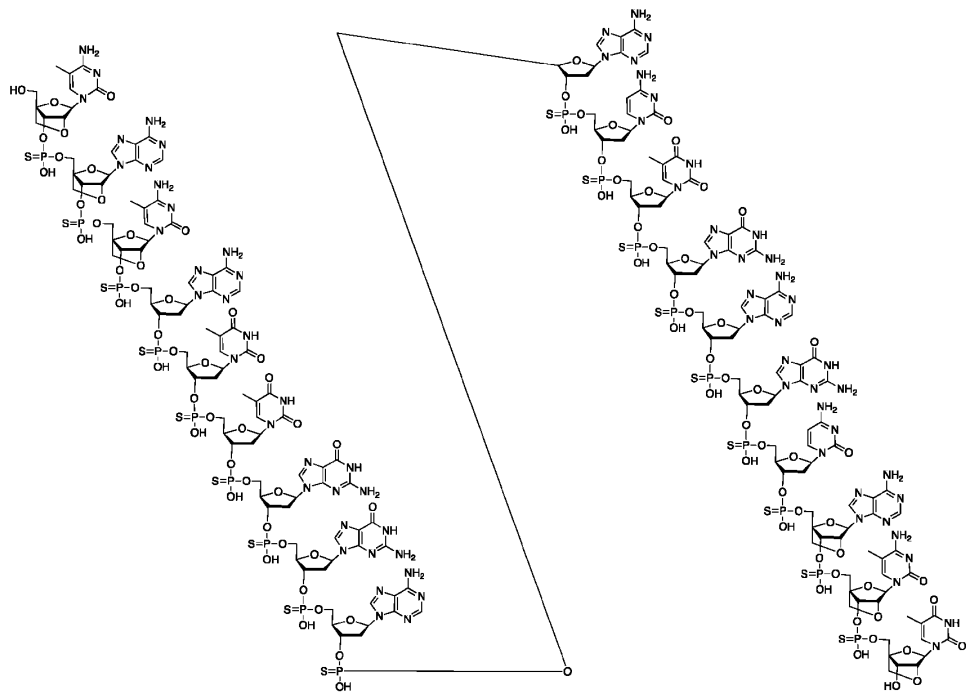
[0103] In certain embodiments, the SNCA ASO from 5' to 3': a 5' wing segment having 3 nucleosides, wherein each nucleoside of the 5' wing segment comprises a modified sugar; a gap segment having from 11 to 13 nucleosides, wherein each nucleoside of the gap segment is a deoxynucleoside; and a 3' wing segment having from 3 nucleosides, wherein each nucleoside of the 3' wing segment comprises a modified sugar. In some embodiments, every internucleotide linkage in the SNCA ASO comprises a PS linkage. In some embodiments, every nucleoside in 5' and 3' wing segments comprises a 2-MOE modified nucleotide, a bicyclic nucleotide, a LNA nucleotide, or a cET modified nucleotide.

[0104] In some embodiments, provided herein is a SNCA ASO comprising or consisting of a modified nucleic acid sequence having at least about 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a modified sequence as shown in **Table 7**. In certain embodiments, the SNCA ASO comprises a modified nucleic acid sequence as shown in **Table 7**. In certain embodiments, the SNCA ASO consists of a modified nucleic acid sequence as shown in **Table 7**. The SNCA ASOs in Table 7 are gapmers having wing segments having LNA modified nucleosides. Similar SNCA ASO gapmers can be made having wing segments having other ribose-modified nucleosides, including, but not limited to, 2'-MOE nucleotides and/or cEt nucleosides.

[0105] In some embodiments, provided herein are modified oligonucleotides according to any of the following chemical structures:

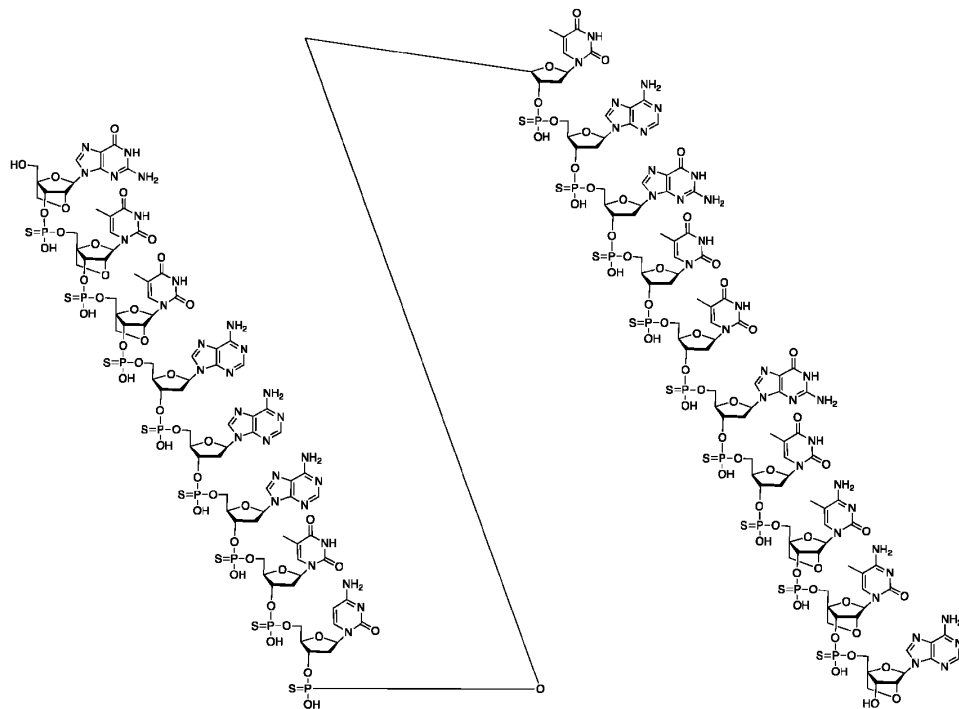
SEQ ID NO: 1: (see Table 7 for key)

+[%C]*+A*+[%C]*dA*dT*dT*dG*dG*dA*dA*dC*dT*dG*dA*dG*dC*+A*+[%C]*+T



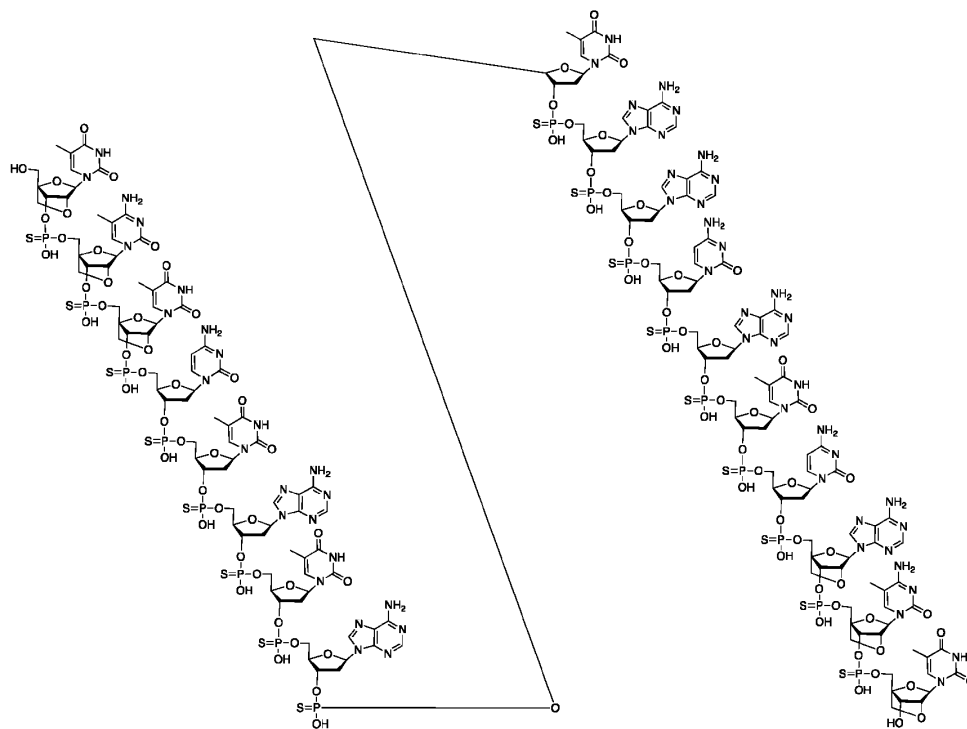
SEQ ID NO: 2: (see Table 7 for key)

+G*+T*+T*dA*dA*dA*dT*dC*dT*dA*dG*dT*dT*dG*dT*+[%C]*+[%C]*+A



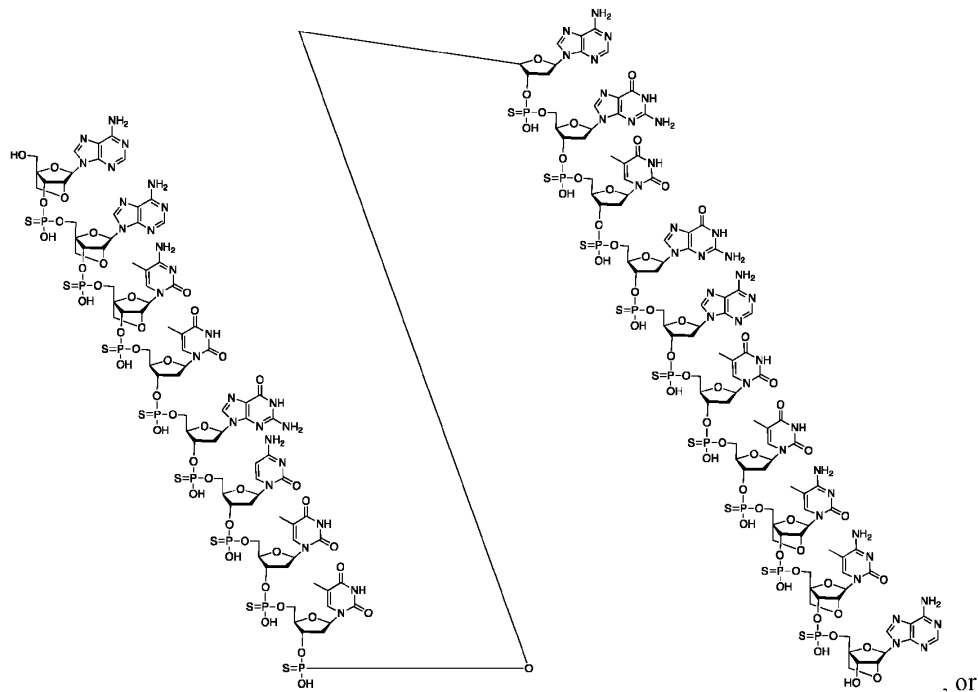
SEQ ID NO: 3: (see Table 7 for key)

+T*+[%C]*+T*dC*dT*dA*dT*dA*dA*dC*dA*dT*dC*+A*+[%C]*+T



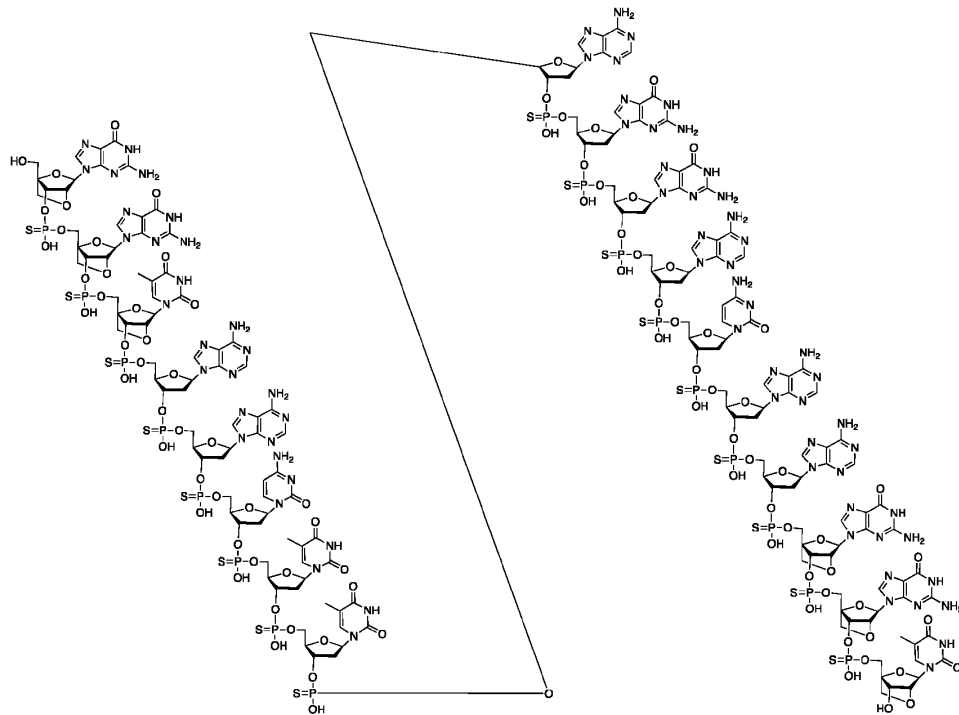
SEQ ID NO: 4: (see Table 7 for key)

+A*+A*+[%C]*dT*dG*dC*dT*dT*dA*dG*dT*dG*dA*dT*dT*+[%C]*+[%C]*+A



SEQ ID NO: 5: (see Table 7 for key)

+G*+G*+T*dA*dA*dC*dT*dT*dA*dG*dG*dA*dC*dA*dA*+G*+G*+T



or a salt thereof.

ASO terminal clipping

[0106] In certain embodiments, SNCA ASOs targeted to a ASyn nucleic acid may be shortened or truncated. In some embodiments, the SNCA ASOs may be shortened or truncated by endonuclease activity. For example, a single subunit (*e.g.*, a nucleotide or a portion thereof) may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a ASyn nucleic acid may have two or more subunits deleted from the 5' end, or alternatively may have two or more subunits deleted from the 3' end, of the SNCA ASO.

II. SNCA ASO CONJUGATES

[0107] Any of the described SNCA ASOs can be linked to a targeting group or a delivery vehicle. The SNCA ASO can be linked to the targeting group or a delivery vehicle directly or indirectly. The SNCA ASO can be linked to the targeting group or a delivery vehicle at the 5' end of the ASO, and the 3' end of the ASO or to any nucleotide in the ASO. The targeting

group or delivery vehicle can be attached (linked) to a sugar moiety, a base moiety, an internucleoside linkage, or a terminus (5' or 3') of the SNCA ASO. Any targeting group or delivery vehicle suitable for facilitating or increasing delivery of a SNCA ASO to a target cell or tissue in a subject known in the art can be conjugated to the SNCA ASO. A targeting group can be, but are not limited to, a molecule that specifically binds to the transferrin receptor (TfR) or a molecule expressed on the luminal surface of the blood brain barrier. The molecule can be, but is not limited to, an anti-TfR antibody or a TfR binding fragment thereof, or a Fc polypeptide modified to bind the TfR. A delivery vehicle can be, but is not limited to, a molecule, a brain shuttle, a protein, a liposome, a lipoplex, or a lipid nanoparticle.

[0108] In some embodiments, a SNCA ASO is linked to a targeting group or delivery vehicle via a linker. Any linker known in the art that is suitable for conjugating a SNCA ASO to another molecule may be used.

III. METHODS OF USE

[0109] The SNCA ASOs may be used for a variety of purposes, including therapeutic indications.

[0110] In some embodiments, methods of reducing the expression of a *SNCA* gene in a subject are described, the methods comprising administering an effective amount of a SNCA ASO, conjugate, or composition as described herein to the subject. In some embodiments, SNCA ASOs, conjugates, or compositions thereof for use in reducing the expression of a *SNCA* gene in a cell of subject are provided. In certain embodiments, administration of a described SNCA ASO to a cell or subject reduces expression of the *SNCA* gene in the cell or subject. Expression can be reduced by more than 10%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, or more than 95%, compared to the expression in a control (*e.g.*, a cell or subject that was not administered the SNCA ASO, conjugate or composition as described herein) or compared to the level of expression of SNCA in the cell or subject prior to administration of the SNCA ASO.

[0111] Certain embodiments provide a method of reducing *SNCA* mRNA expression in a subject in need thereof, the method comprising administering to the subject a SNCA ASO, conjugate or composition as described herein.

[0112] Certain embodiments also provide a method of reducing ASyn expression in a subject in need thereof, the method comprising administering to the subject a SNCA ASO, conjugate or composition as described herein.

[0113] In certain embodiments, a SNCA ASO as described herein may be used to reduce SNCA expression in a brain cell. The brain cell can be, but is not limited to a neuron, an oligodendrocyte, a deep brain cell, a cell of the hippocampus, or a cell of the entorhinal cortex. Thus, certain embodiments provide a method of generating a neuron cell or an oligodendrocyte with decreased ASyn expression, the method comprising delivering to the neuron cell or oligodendrocyte a SNCA ASO, a conjugate or a composition as described herein, wherein the SNCA ASO decreases the expression level of a *SNCA* gene (*e.g.*, an endogenous *SNCA* gene). Certain embodiments also provide a method of modifying a neuron cell or an oligodendrocyte to decrease ASyn expression, the method comprising delivering to the neuron cell oligodendrocyte a SNCA ASO, a conjugate or a composition as described herein, wherein the SNCA ASO decreases the expression level of a *SNCA* gene (*e.g.*, an endogenous *SNCA* gene). In certain embodiments, the SNCA ASO binds to a *SNCA* transcript and reduces the expression level of a *SNCA* transcript in the cell. In certain embodiments, the SNCA ASO binds to a *SNCA* transcript and recruits RNase H, which degrades the transcript.

[0114] Reducing SNCA expression includes reducing transcription of a *SNCA* transcript or mRNA, knockdown of *SNCA* transcript or mRNA, reducing expression of the *SNCA* gene, or reducing the level of SNCA protein.

[0115] In certain embodiments, a SNCA ASO as described herein may be used to reduce SNCA expression in a spinal cord or a cell of the spinal cord.

[0116] In certain embodiments, a SNCA ASO as described herein is delivered to a cell within the brain of a subject, such as a neuron, a glial cell, an oligodendrocyte, a deep brain cell, a cell of the hippocampus, or a cell of the entorhinal cortex.

[0117] Certain embodiments provide a method of delivering a SNCA ASO to the CNS of a subject in need thereof, comprising administering to the subject a SNCA ASO, a conjugate, or a composition as described herein, wherein said SNCA ASO decreases the expression level of a *SNCA* gene (*e.g.*, an endogenous *SNCA* gene).

[0118] In certain embodiments, the SNCA ASO decreases the expression level of an endogenous *SNCA* gene or reduces the level of a *SNCA* transcript by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% as compared to the level *SNCA* transcript in the absence of administering the SNCA ASO. In certain embodiments, the expression of an endogenous *SNCA* gene or level of a *SNCA* transcript is reduced by at least about 50% relative to the level of expression prior to administration of the SNCA ASO. In certain embodiments, the expression of an endogenous *SNCA* gene or level of

SNCA transcript is reduced by at least about 70% relative to the level of expression prior to administration of the SNCA ASO.

[0119] The SNCA ASOs, and conjugates and compositions containing a SNCA ASOs, described herein may also be used to treat, prevent, or ameliorate a disease, disorder, and condition associated with aSyn. Thus, in some embodiments, provided herein are methods of treatment, prevention, or amelioration of diseases, disorders, and conditions associated with aSyn in a subject in need thereof. In certain embodiments, a disease, disorder, and condition associated with aSyn is an aSyn-associated neurodegenerative disorder (*i.e.*, a synucleinopathy). In certain embodiments, Synucleinopathies include, but are not limited to, Parkinson's Disease, dementia with Lewy bodies (DLB), multiple system atrophy (MSA), PAF, and RBD.

[0120] Accordingly, certain embodiments provide methods of treating an aSyn-associated neurodegenerative disorder in a subject in need thereof, the method comprising administering to the subject a SNCA ASO, or a conjugate or composition containing a SNCA ASO as described herein.

[0121] In certain embodiments, the aSyn-associated neurodegenerative syndrome is Parkinson's Disease. Thus, certain embodiments provide a method of treating Parkinson's disease, the method comprising administering to a subject in need thereof, a SNCA ASO, or a conjugate or composition containing a SNCA ASO, as described herein. The subject may be diagnosed with Parkinson's disease, diagnosed with one or more symptoms of Parkinson's disease, or be at risk of developing Parkinson's disease or one or more symptoms associated with Parkinson's disease.

[0122] In certain embodiments, the subject is a human subject.

[0123] A SNCA ASO, or a conjugate or composition containing a SNCA ASO, as described herein may be administered to a subject at a therapeutically effective amount or dose. The dosages, however, may be varied according to several factors, including the chosen route of administration, the formulation of the composition, patient response, the severity of the condition, the subject's weight, and the judgment of the prescribing physician. The dosage can be increased or decreased over time, as required by an individual patient.

[0124] In some embodiments, a SNCA ASO, or a conjugate or composition containing a SNCA ASO, as described herein is administered parenterally. In some embodiments, the SNCA ASO, conjugate, or composition is administered intravenously. Intravenous administration can be by infusion, *e.g.*, over a period of from about 5 to about 10 minutes, about 10 to about 30 minutes, or over a period of at least 1 hour, 2 hours, or 3 hours. In some

embodiments, the SNCA ASO, conjugate, or composition, is administered as an intravenous bolus. Combinations of infusion and bolus administration may also be used.

[0125] In some parenteral embodiments, a SNCA ASO, or a conjugate or composition containing a SNCA ASO, is administered intraperitoneally, subcutaneously, intradermally, or intramuscularly. In some embodiments, the SNCA ASO, conjugate, or composition is administered intradermally or intramuscularly. In some embodiments, the SNCA ASO, conjugate, or composition is administered intrathecally, such as intracerebroventricularly into the lateral ventricle.

[0126] In other embodiments, a SNCA ASO, or a conjugate or composition containing a SNCA ASO, as described herein may be administered orally, by pulmonary administration, intranasal administration, intraocular administration, or by topical administration. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

IV. PHARMACEUTICAL COMPOSITIONS AND KITS

[0127] In some embodiments, pharmaceutical compositions and kits comprising a SNCA ASO or conjugate as described herein are provided.

Pharmaceutical compositions

[0128] Guidance for preparing formulations for use as described herein can be found in any number of handbooks for pharmaceutical preparation and formulation that are known to those of skill in the art.

[0129] In some embodiments, a pharmaceutical composition comprises a SNCA ASO or conjugate thereof as described herein and further comprises one or more pharmaceutically acceptable carriers, diluents, and/or excipients.

[0130] As used herein, the term pharmaceutically acceptable carrier includes any solvents, dispersion media, or coatings that are physiologically compatible and that preferably do not interfere with or otherwise inhibit the activity of the active agent. Various pharmaceutically acceptable excipients are well-known. In some embodiments, the carrier is suitable for intravenous, intrathecal, intracerebroventricular, intramuscular, oral, intraperitoneal, transdermal, topical, or subcutaneous administration. Pharmaceutically acceptable carriers can contain one or more physiologically acceptable compounds that act, for example, to stabilize the composition or to increase or decrease the absorption of the SNCA ASO or conjugate. Physiologically acceptable compounds can include, for example, carbohydrates (*e.g.*, glucose, sucrose, or dextrans), antioxidants (*e.g.*, ascorbic acid or glutathione), chelating agents, low

molecular weight proteins, compositions that reduce the clearance or hydrolysis of the active agents, excipients, or other stabilizers and/or buffers. Other pharmaceutically acceptable carriers and their formulations are also available in the art.

[0131] The pharmaceutical compositions described herein can be manufactured in a manner that is known to those of skill in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, emulsifying, encapsulating, entrapping, or lyophilizing processes. The following methods and excipients are merely exemplary and are in no way limiting.

[0132] For oral administration, a SNCA ASO or conjugate as described herein can be formulated by combining it with one or more pharmaceutically acceptable carriers that are known in the art. In some embodiments, a pharmaceutically acceptable carrier can be used to formulate a compound (*e.g.*, SNCA ASO or conjugate) as a tablet, pill, dragee, capsule, emulsion, lipophilic and hydrophilic suspension, liquid, gel, syrup, slurry, suspension or the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by mixing the SNCA ASOs or conjugates with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone. If desired, disintegrating agents can be added, such as a cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0133] As disclosed above, a SNCA ASO or conjugate as described herein can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. For injection, the SNCA ASOs or conjugates can be formulated into preparations by dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers, and preservatives. In some embodiments, SNCA ASOs or conjugates can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions can

take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

[0134] For injection administration, a SNCA ASO or conjugate as described herein can be formulated by combining it with one or more pharmaceutically acceptable carriers that are known in the art. In some embodiments, a pharmaceutically acceptable carrier can be used to formulate a compound (e.g., SNCA ASO or conjugate) as an emulsion, lipophilic and hydrophilic suspension, liquid, suspension or the like, for injection into a patient to be treated. In some embodiments, a SNCA ASO or SNCA ASO conjugate is formulated for intravenous injection, intrathecal injection, intraarterial injection, or intracerebroventricular injection.

[0135] For intranasal administration, a SNCA ASO or conjugate as described herein can be formulated by combining it with one or more pharmaceutically acceptable carriers that are known in the art to be suitable of intranasal administration of a drug (Shah P et al. "Intranasal delivery: An attractive route for the administration of nucleic acid based therapeutics for CNS disorders" Front Pharmacol. 2022 Aug 30;13:974666.)

[0136] Typically, a pharmaceutical composition for use in *in vivo* administration is sterile. Sterilization can be accomplished according to methods known in the art, e.g., heat sterilization, steam sterilization, sterile filtration, or irradiation.

[0137] Dosages and desired drug concentration of pharmaceutical compositions as described herein may vary depending on the particular use envisioned.

Kits

[0138] In some embodiments, kits comprising a SNCA ASO or conjugate as described herein are provided. In some embodiments, the kits are for use in reducing the SNCA expression.

[0139] The described SNCA ASOs (including SNCA ASO conjugates) and pharmaceutical compositions comprising SNCA ASOs disclosed herein may be packaged or included in a kit, container, pack, or dispenser. The SNCA ASOs and pharmaceutical compositions comprising said SNCA ASOs may be packaged in pre-filled syringes or vials. Any of the SNCA ASOs or pharmaceutical compositions containing a SNCA ASO identified herein can be formulated or packaged in single-dose or multi-dose format. Any of the SNCA ASOs or pharmaceutical compositions containing a SNCA ASO identified herein can be formulated for repeat dosing.

[0140] In some embodiments, the kit further comprises instructional materials containing directions (*i.e.*, protocols) for the practice of the methods described herein (e.g., instructions for using the kit for administering a composition across the blood-brain barrier). While the instructional materials typically comprise written or printed materials, they are not limited to

such. Any medium capable of storing such instructions and communicating them to an end user is contemplated herein. Such media include, but are not limited to, electronic storage media (*e.g.*, magnetic discs, tapes, cartridges, chips), optical media (*e.g.*, CD-ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

EXAMPLES

[0141] The present invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes only, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, *etc.*), but some experimental error and deviation may be present. The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature.

Example 1: In Vitro Screening of ASOs to the *SNCA* gene

[0142] Selection of ASO sequences specific for a given target sequence is based upon analysis of the chosen target sequence and determination of a number of factors, including *in vitro* and *in vivo* potency, and liver toxicity profile.

Initial SNCA ASO Screening

[0143] Initial identification of 309 SNCA ASO sequences 100% complementary to the SNCA pre-mRNA (Ensembl ENST00000394991) (SEQ ID NO: 48) and SNCA mRNA (refseq NM_000345) (SEQ ID NO: 49) were made based on computational predictions of target binding, specificity, efficacy and safety.

[0144] Knockdown efficacy of the SNCA-specific ASOs was tested in human HeLa and EFO-21 cells. The cells were treated with SNCA-specific ASO or control oligonucleotide at a concentration of 5 μ M without the use of a transfection reagent. After three days treatment, cells were lysed. SNCA and HPRT1 mRNA expression was analyzed using the QuantiGene Singleplex assay (ThermoFisher). SNCA expression values were normalized to HPRT1 values and the degree of knockdown in comparison to mock-treated cells was determined. 22 ASOs were selected for investigation of the TLR9 activating capacity and concentration response relationship (IC50 determination).

Investigation of the TLR9 activating capacity

[0145] 22 ASOs were tested for their TLR9-dependent proinflammatory potential. Therefore, a reporter cell line (HEK-Blue-hTLR9 cells, Invivogen) was treated with *SNCA*-specific ASOs, a positive and a negative control without the use of transfection reagent at a concentration of 5 μ M. After 20h, QUANTI-Blue™ Solution (Invivogen) was added to the cells. Optical density was measured 2 hours later in order to determine the TLR9 activating capacity of the tested ASOs.

In Vitro IC50 Determination

[0146] Investigation of concentration-response relationship and determination of half maximal inhibitory concentration (IC50) values was performed in EFO-21 cells for 22 selected *SNCA*-specific ASOs. Cells were treated with the *SNCA*-specific ASOs at the following concentrations: 5000, 1667, 556, 185, 62, 21, 7 nM without the addition of a transfection reagent. After three days treatment, cells were lysed. *SNCA* and HPRT1 mRNA expression was analyzed using the QuantiGene Singleplex assay (Thermo Fisher). *SNCA* expression values were normalized to HPRT1 values and the degree of knockdown in comparison to mock-treated cells was determined. Results are shown in **Table 5** below.

Table 5

ASO ID for Patent	IC ₅₀ [nM]	R ²
ASO_B1	n.d.	0.74
ASO_B2	640	0.82
ASO_B3	3658	0.85
ASO_S2	1193	0.88
ASO_B4	1796	0.57
ASO_S3	409	0.90
ASO_B5	676	0.90
ASO_B6	1554	0.90
ASO_S4	712	0.88
ASO_S5	497	0.95
ASO_B7	848	0.94
ASO_B8	410	0.93
ASO_B9	1871	0.84
ASO_B10	1000	0.88
ASO_B11	607	0.83
ASO_B12	1739	0.91
ASO_S6	817	0.81
ASO_B13	802	0.76
ASO_B14	1592	0.89
ASO_S7	830	0.92

ASO_B15	n.d.	0.86
ASO_B16	339	0.91
ASO_S1 (Control)	982	0.97

[0147] Six sequences, ASO_S2, ASO_S3, ASO_S4, ASO_S5, ASO_S6, and ASO_S7, were selected based on the combination of based on in vitro potency assessment and lack of significant liver toxicity in mice for further in vivo safety and potency analysis.

Example 2: In Vivo Knockdown Screening

[0148] The six ASOs selected above (based on in vitro potency assessment and lack of significant liver toxicity in mice) and control ASO_1 were diluted in sterile saline and administered to hSNCA (mSnca^{-/-}) mice via ICV injection at 50 µg (ASO_S1, ASO_S2, ASO_S3, ASO_S4, ASO_S5, ASO_S7) or 25 µg due to acute neurotoxicity (ASO_S6). All ASOs were dosed at a final volume of 10 µL. Two weeks post dose, tissues were harvested and SNCA expression was measured in the brain and spinal cord via bulk RNA isolation followed by qPCR of *SNCA* and *Gapdh* according to the protocol described below. *SNCA* knockdown in cortex and spinal cord are shown in FIG. 1A and FIG. 1B, respectively. *Gfap* and *Itgax* expression were measured in the brain in the same manner to assess glial activation in response to ASO deposition (FIG 1C). ASO_S2, ASO_S3, ASO_S4, ASO_S5, and ASO_S6 were selected for further investigation. ASO_S7 was eliminated due to low potency along with induced glial activation.

[0149] ASO concentrations were also measured for ASO_S2, S ASO_3, ASO_S4 and ASO_S5 as described below in brain to confirm consistent dosing (FIG. 3). The data in FIG. 1 demonstrate that ASO_S2, ASO_S3, ASO_S4, ASO_S5, and ASO_S6 show significant reduction of hSNCA when delivered to the brain of mice at high concentrations.

Example 3: In Vivo Liver Toxicity Screening

[0150] Liver safety was assessed in mice for each ASO by measuring serum ALT, AST, and LDH levels. ASO_S2, ASO_S3, ASO_S4, ASO_S5, and ASO_S6 were administered by subcutaneous injection to wildtype mice at 20 mpk daily for five consecutive days. No significant liver toxicities were observed in mice with any of the ASOs.

Example 4: In Vivo ED50 Screening

[0151] To assess potency of the ASOs, ASO_S1 control and ASO_S2, S ASO_3, ASO_S4, ASO_S5, and ASO_S6 were diluted in sterile saline and administered to hSNCA (mSNCA^{-/-}) mice via ICV injection at 10 µg (ASO_S1, ASO_S2, ASO_S3, ASO_S4, ASO_S5, ASO_S6)

and 25 μ g (ASO_S1, ASO_S2, ASO_S3, ASO_S4, ASO_S5). All ASOs were dosed at a final volume of 10 μ L. Two weeks post dose, tissues were harvested and SNCA expression was measured in the brain and spinal cord via bulk RNA isolation followed by qPCR of *SNCA* and *Gapdh* according to the protocol described below. SNCA knockdown in cortex and spinal cord are shown in FIG. 2A and FIG. 2B, respectively. Using data from all doses (10, 25, and 50 μ g when available), ED50 values were calculated using Graphpad Prism software (FIG. 2A and FIG. 2B). The data in FIG. 2 demonstrate that ASOs ASO_S2, ASO_S3, ASO_S4, ASO_S5, and ASO_S6 are highly potent in hSNCA mouse CNS, with ED50 values as low as $\sim$ 11 μ g.

Table 6. SNCA knockdown

ASO	Brain ED50 (μ g)	Spinal Cord ED50 (μ g)
ASO_S1	16.02	12.22
ASO_S2	62.22	54.05
ASO_S3	11.58	11.76
ASO_S4	23.86	19.36
ASO_S5	19.9	17.21
ASO_S6	28.9	13.45

Example 6: In Vivo Rat Renal Toxicity Determination

[0152] All ASOs (*i.e.*, ASO_S2, ASO_S3, ASO_S4, ASO_S5, and ASO_S6) were well tolerated in Wistar Hannover rats in a 2-week repeat dose study (two 40 mg/kg subcutaneous injections of ASO on Day 1 and 8) with no changes in clinical signs or body weight throughout the treatment period. Serum blood urea nitrogen (BUN) and creatinine levels (markers of kidney function) were within normal historical range in all treatment groups and comparable to vehicle controls. On Day 15, changes in urine KIM-1: creatinine ratio (index for renal injury) were considered below critical limit and of a magnitude of change commonly observed in rats under similar study conditions. In conclusion, no evidence of adverse effects on kidney function or histopathology were detected in mice dosed with the ASOs.

In Vivo Methods

[0153] *Tissue homogenization for RNA measurements.* Weighed frozen tissue samples were processed for RNA assays by adding 10 $\times$ volume Qiazol reagent. Samples were homogenized using 5 mm tungsten carbide beads in 2mL Eppendorf tubes, shaken using the Qiagen TissueLyzer II (Cat No./ID: 85300) (2 $\times$ 3 min at 27 Hz). After lysis, samples were incubated for 5min at room temperature, then chloroform was added. Samples were vortexed,

incubated at room temperature for 3 min, then centrifuged for 15min at 12000×g at 4°C. The aqueous phase was then isolated. RNA was then isolated by adding isopropanol, vortexing, incubating for 10 minutes at room temperature, then centrifuging for 10 min at 12000×g at 4°C. The resulting pellet was then resuspended in 75% ethanol, vortexed and centrifuged for 5min at 7500×g at 4°C. The final pellet was resuspended in water.

[0154] *qPCR analysis.* To evaluate target mRNA levels, qRT-PCR was run on RNA extracted from tissue lysates. Target mRNA levels were evaluated using Taqman probes (hSNCA, mGapdh) and the Express One-Step Kit. For each sample, hSNCA mRNA levels were normalized to the housekeeping gene Gapdh. The same approach was used to measure glial activation markers Gfap and Itgax. qRT-PCR was performed using a QuantStudio 6 Flex system (Applied Biosystems) and average CT values were measured for each probe using technical duplicates. Next, the delta delta CT values were calculated relative to the non-ASO treated group and plotted as relative expression levels.

[0155] *Tissue homogenization for drug concentration measurement.* Weighed frozen tissue samples were processed for biochemical assays by adding 10× volume chilled 1% NP40 + PBS homogenization buffer with added cOmplete Protease Inhibitor (Roche #04693132001) and PhosStop (Roche 04906837001) phosphatase inhibitors. Samples were homogenized using 3 mm tungsten carbide beads in 1.5mL Eppendorf tubes, shaken using the Qiagen TissueLyzer II (Cat No./ID: 85300) (2×3 min at 27 Hz).

[0156] *Total ASO Assay.* Quantification of total ASO (in conjugated and free forms) in mouse plasma and tissue homogenates were measured using a hybridization-based electrochemiluminescence immunoassay (ECLIA). Briefly, custom biotinylated and digoxigenin-conjugated antisense probes (synthesized by Integrated DNA Technologies, Coralville, IA) at working concentrations were combined with prepared test samples (with sample pre-dilution, where appropriate) and relevant standards in TE Buffer (10 mM Tris-HCL containing 1mM EDTA). Prepared samples in TE buffer were added, in a 1:1 mix, into 1× SSC Buffer (Sigma-Aldrich, St. Louis, MO) containing a working concentration of recombinant proteinase K enzyme (ThermoFisher, Waltham, MA). Hybridization/Enzyme mixture was then digested, detantured, annealed, and cooled in a thermal cycler instrument. Following hybrid product incubation, samples were added to the wells of an MSD GOLD 96-well streptavidin-coated microtiter plate (Meso Scale Discovery, Rockville, MD) and incubated for approximately 30 mins. Following incubation and a plate wash step, secondary ruthenylated (SULFO-TAG) sheep anti-digoxigenin antibody (Novus Biologicals, Littleton, CO) at a working concentration in assay diluent was added to the plate and incubated for approximately

30 mins. Following a plate wash, a 1× MSD Read Buffer T (Meso Scale Discovery, Rockville, MD) was then added to generate the electrochemiluminescence (ECL) assay signal, which was then expressed in ECL units (ECLU). All of the assay reaction steps were performed at ambient temperature with shaking on a plate shaker (where appropriate); and all test samples were pre-diluted at the assay MRD of 1:20 prior to analyzing in the assay plate. Sample ECLU signals generated in the assay subsequently were processed into concentrations by back-calculating off the assay calibration (CS) curve. The assay CS curve was fitted with a weighted four-parameter nonlinear logistic regression for use in calculating concentrations for unknown/test samples.

[0157] *Intracerebroventricular Bolus (ICV) surgery.* Followed procedures from DeVos, J Vis Exp (2013) herein incorporates by reference and summarized in brief as follows: in preparation for the surgery, the surgical area was sterilized with 70% ethanol. Mice were brought under anesthesia with 4% isoflurane. Hair was shaved between from the shoulder region to between the eyes prior to placing mouse on stereotax surface. With a maintenance level of 2% isoflurane, an incision was made from the base of the neck up to between the eyes. Following cleaning with hydrogen peroxide, a needle was slowly driven through the skull at a rate of 1 mm/s. After a 2–3-minute period to allow for brain sealing around the needle, a dose of 10 µl ASO at 1 µl per second was administered. With a cotton swab held against the skull at the base of the needle, the needle was raised at a rate of 1 mm per second. The cotton swab was held at the site of injection for 1 minute to limit drug leakage. Following ICV bolus, the incision was sutured and treated with an antibiotic ointment. The mouse was transferred to a headed recovery pad and observed for full recovery. Mice were monitored daily after surgery to check for pain, discomfort, or infections.

Table 7

Name	SEQ ID NO	Corresponding Modified Sequence**
ASO_S2	1	5' +[%C]*+A*+[%C]*dA*dT*dT*dG*dG*dA*dA*dC*dT*dG*dA*dG*dC*+A*+[%C]*+T* 3'
ASO_S3	2	5' +G*+T*+T*dA*dA*dA*dT*dC*dT*dA*dG*dT*dT*dG*dT*+[%C]*+[%C]*+A 3'
ASO_S4	3	5' +T*+[%C]*+T*dC*dT*dA*dT*dA*dT*dA*dT*dA*dA*dC*dA*dT*dC*+A*+[%C]*+T 3'
ASO_S5	4	5' +A*+A*+[%C]*dT*dG*dC*dT*dT*dA*dG*dT*dG*dA*dT*dT*+[%C]*+[%C]*+A 3'
ASO_S6	5	5' +G*+G*+T*dA*dA*dC*dT*dT*dA*dG*dG*dA*dC*dA*dA*+G*+G*+T 3'
ASO_S1 control	47	5' +A*dT*dT*+[%C]*dC*dT*dT*dT*dA*dC*dA*dC*dC*+A*+[%C]*dA*+[%C]*+T 3'
** + (("[%C]", +A, +T, +G) indicate an LNA nucleoside d (dA, dC, dG, and dT) indicates a deoxyribonucleotide [%C] indicates a 5-methylcytosine nucleoside * indicates a phosphorothioate (PS) internucleoside linkage		

Claims:

1. A SNCA antisense oligonucleotide (SNCA ASO) comprising a nucleic acid sequence that comprises at least 9 contiguous nucleobases of a sequence selected from any one of SEQ ID NOs: 1-15 and 18-23 and at least one modified internucleoside linkage and/or at least one modified sugar.
2. The SNCA ASO of claim 1, wherein the SNCA ASO consists of 15 to 25 linked nucleosides.
3. The SNCA ASO of claim 1, wherein the SNCA ASO is 16-20 nucleotides in length.
4. The SNCA ASO of claim 1, wherein the SNCA ASO is 16 nucleotides in length.
5. The SNCA ASO of claim 1, wherein the SNCA ASO comprises the sequence of any one of SEQ ID NOs: 24-38 and 41-46.
6. The SNCA ASO of any one of claims 1-5, wherein the nucleic acid sequence is at least 90% complementary to SEQ ID NO: 49.
7. The SNCA ASO of any one of claims 1-6, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.
8. The SNCA ASO of any one of claims 1-7, wherein the at least one modified sugar is a bicyclic sugar.
9. A SNCA ASO comprising a nucleic acid sequence as set forth in any one of SEQ ID NOs: 1-15, 18-38, and 41-46 that comprises at least one modified internucleoside linkage and/or at least one modified sugar.
10. The SNCA ASO of claim 9, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.
11. The SNCA ASO of claim 9 or 10, wherein the at least one modified sugar is a bicyclic sugar.
12. The SNCA ASO of claim 11, wherein each bicyclic sugar comprises a chemical bridge between the 4' and 2' positions of the sugar, wherein each chemical bridge is

independently selected from: 4'-CH(R)-O-2' and 4'-(CH₂)₂-O-2', wherein each R is independently selected from H, C₁-C₆ alkyl and C₁-C₆ alkoxy.

13. The SNCA ASO of claim 12, wherein each bicyclic sugar comprises a chemical bridge between the 4' and 2' positions of the sugar, wherein each chemical bridge is 4'-CH(R)-O-2', and wherein each R is independently H.

14. The SNCA ASO of any one of claims 9-13, further comprising a modified nucleobase.

15. The SNCA ASO of claim 14, wherein the modified nucleobase is a 5-methyl cytosine.

16. The SNCA ASO of any one of claims 9-15 comprising from 5' to 3':

a 5' wing segment having from 1 to 6 nucleosides, wherein each nucleoside of the 5' wing segment comprises a modified sugar;

a gap segment having from 8 to 15 nucleosides, wherein each nucleoside of the gap segment is a deoxynucleoside; and

a 3' wing segment having from 1 to 6 nucleosides, wherein each nucleoside of the 3' wing segment comprises a modified sugar.

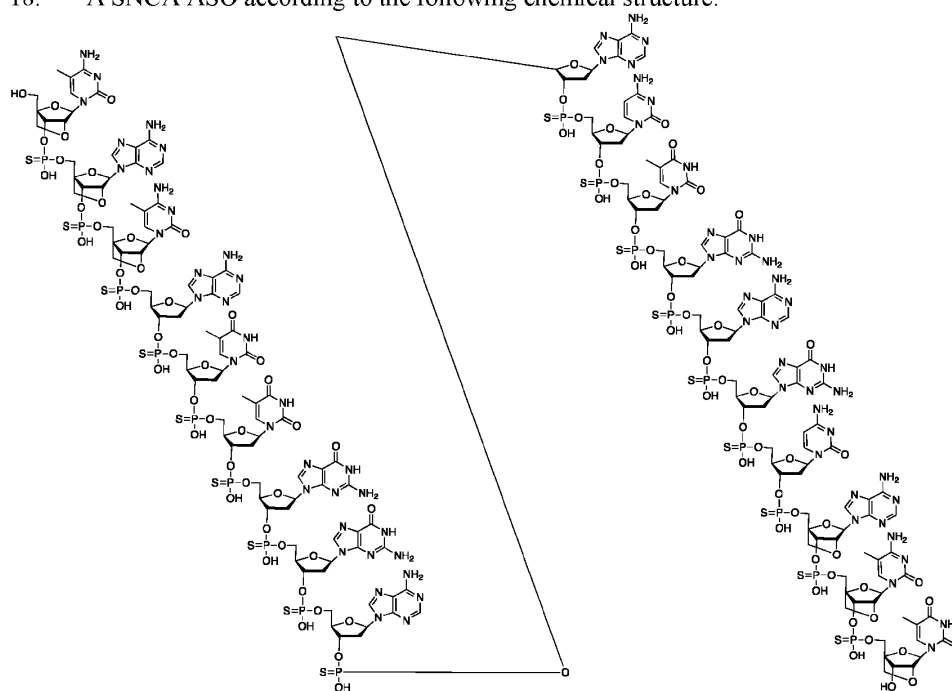
17. The SNCA ASO of any one of claims 9-16 comprising from 5' to 3':

a 5' wing segment having 3 nucleosides, wherein each nucleoside of the 5' wing segment comprises a modified sugar;

a gap segment having from 11 to 13 nucleosides, wherein each nucleoside of the gap segment is a deoxynucleoside; and

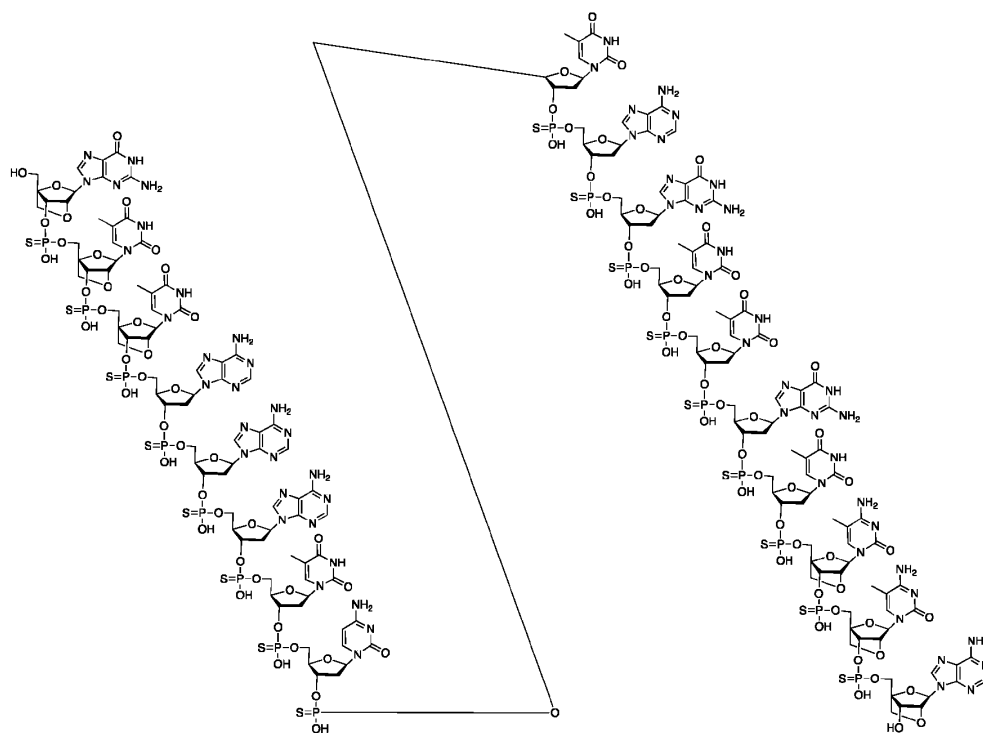
a 3' wing segment having 3 nucleosides, wherein each nucleoside of the 3' wing segment comprises a modified sugar.

18. A SNCA ASO according to the following chemical structure:



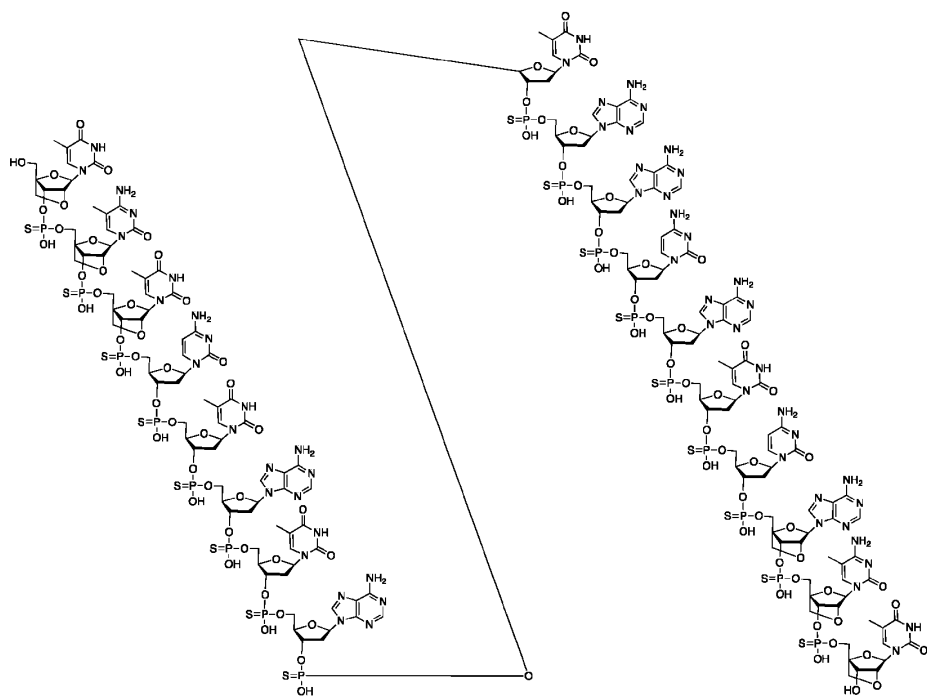
or a salt thereof.

19. A SNCA ASO according to the following chemical structure:



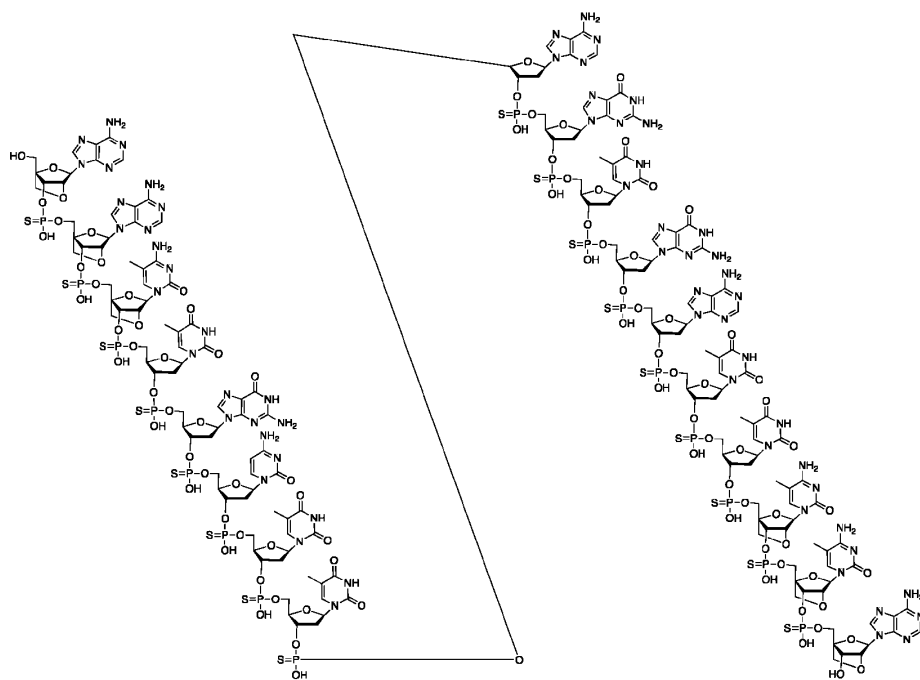
or a salt thereof.

20. A SNCA ASO according to the following chemical structure:



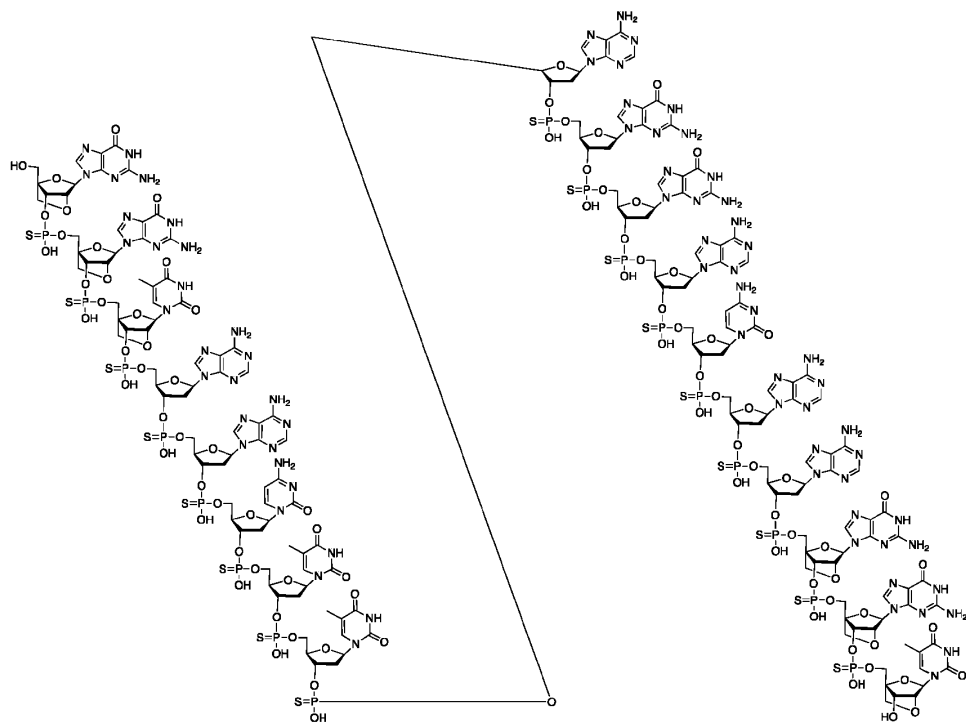
or a salt thereof.

21. A SNCA ASO according to the following chemical structure:



or a salt thereof.

22. A SNCA ASO according to the following chemical structure:



or a salt thereof.

23. The SNCA ASO of any one of claims 1-22, wherein the SNCA ASO is conjugated to a targeting ligand or a delivery vehicle.

24. The SNCA ASO of claim 23, wherein the targeting ligand specifically binds to the transferrin receptor (TfR) or a molecule expressed on the luminal surface of the blood brain barrier.

25. A pharmaceutical composition comprising a SNCA ASO of any one of claims 1-24 and a pharmaceutically acceptable carrier or diluent.

26. A method of generating a neuron cell, a glial cell, or an oligodendrocyte with decreased alpha-synuclein expression, the method comprising delivering to the neuron cell, the glial cell, or oligodendrocyte a SNCA ASO of any one of claims 1-24, wherein the SNCA ASO decreases the expression level of an endogenous *SNCA* gene.

27. A method of modifying a neuron cell, a glial cell, or an oligodendrocyte to decrease alpha-synuclein expression, the method comprising delivering to the neuron cell, the glial cell, or the oligodendrocyte a SNCA ASO of any one of claims 1-24, wherein the SNCA ASO decreases the expression level of an endogenous *SNCA* gene.
28. A method of modifying a neuron cell, a glial cell, or an oligodendrocyte to decrease alpha-synuclein expression, the method comprising delivering to the neuron cell, the glial cell, or the oligodendrocyte a SNCA ASO of any one of claims 1-24, wherein the SNCA ASO specifically reduces the expression level of a *SNCA* transcript in the cell.
29. The method of claim 28, wherein the SNCA ASO binds to a *SNCA* transcript and recruits RNase H which degrades the transcript.
30. A method of reducing expression of alpha-synuclein in a spinal cord cell of a subject comprising administering the SNCA ASO of any one of claims 1-24 or the pharmaceutical composition of claim 25 by intrathecal administration.
31. A method of reducing alpha-synuclein expression in a subject comprising administering the SNCA ASO of any one of claims 1-23 or the pharmaceutical composition of claim 25 to the subject.
32. The method of claim 31, wherein alpha-synuclein expression is reduced in the CNS of the subject.
33. The method of claim 31, wherein the SNCA ASO is administered to the subject by intrathecal administration.
34. The method of any one of claims 26-33 wherein the SNCA ASO decreases the expression level of an endogenous *SNCA* gene or reduces the level of a *SNCA* transcript by at least about 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% as compared to the level without administering the SNCA ASO.
35. The method of claim 34, wherein the expression of the endogenous *SNCA* gene or the level of the *SNCA* transcript is reduced by at least about 50%.

36. The method of claim 34, wherein the expression of an the endogenous *SNCA* gene or the level of the *SNCA* transcript is reduced by at least about 70%.
37. The method of any one of claims 31-36, wherein the SNCA ASO is administered to the subject intravenously.
38. A method of treating an alpha-synuclein-associated neurodegenerative disorder in a human subject in need thereof, the method comprising administering to the human subject the SNCA ASO of any one of claims 1-24 or the pharmaceutical composition of claim 25.
39. The method of claim 38, wherein the alpha-synuclein-associated neurodegenerative syndrome is Parkinson's Disease.
40. The method of claim 38, wherein the alpha-synuclein-associated neurodegenerative syndrome is multiple system atrophy.
41. A method of treating Parkinson's disease, the method comprising administering to a human subject in need thereof, the pharmaceutical composition of claim 25.
42. A method of treating multiple system atrophy, the method comprising administering to a human subject in need thereof, the pharmaceutical composition of claim 25.
43. A method of reducing *SNCA* messenger ribonucleic acid (mRNA) expression in a human subject in need thereof, the method comprising administering to the human subject the SNCA ASO of any one of claims 1-24 or the pharmaceutical composition of claim 25.
44. The pharmaceutical composition as described in claim 25, for use in delivering a SNCA ASO to the CNS of a human subject in need thereof, wherein said SNCA ASO decreases the expression level of an endogenous *SNCA* gene.
45. The pharmaceutical composition as described in claim 25 for use in treating an alpha-synuclein-associated neurodegenerative disorder in a human subject in need thereof.
46. A pharmaceutical composition as described in claim 45, wherein the alpha-synuclein-associated neurodegenerative disorder is Parkinson's.
47. A pharmaceutical composition as described in claim 45, wherein the alpha-synuclein - associated neurodegenerative disorder is multiple system atrophy.

48. The pharmaceutical composition as described in claim 25 for use in reducing *SNCA* mRNA expression in a human subject in need thereof.