

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2015231130 C1**

(54) Title
Compositions for modulating Ataxin 2 expression

(51) International Patent Classification(s)
C12N 15/113 (2010.01)

(21) Application No: **2015231130**

(22) Date of Filing: **2015.03.19**

(87) WIPO No: **WO15/143246**

(30) Priority Data

(31) Number
61/982,131
61/955,705

(32) Date
2014.04.21
2014.03.19

(33) Country
US
US

(43) Publication Date: **2015.09.24**

(44) Accepted Journal Date: **2021.06.10**

(44) Amended Journal Date: **2021.12.23**

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(56) Related Art
I. BEZPROZVANNY ET AL, "Therapeutic prospects for spinocerebellar ataxia type 2 and 3", DRUGS OF THE FUTURE, ES, (2009-01-01), vol. 34, no. 12, doi:10.1358/dof.2009.034.12.1443434, ISSN 0377-8282, pages 1 - 17
WO 2004047872 A2
US 20050209178 A1
WO 1997/042314 A1



- (51) **International Patent Classification:**
C12N 15/113 (2010.01)
- (21) **International Application Number:**
PCT/US2015/021608
- (22) **International Filing Date:**
19 March 2015 (19.03.2015)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/955,705 19 March 2014 (19.03.2014) US
61/982,131 21 April 2014 (21.04.2014) US
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(81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) **Title:** COMPOSITIONS FOR MODULATING ATAXIN 2 EXPRESSION

(57) **Abstract:** Disclosed herein are antisense compounds and methods for decreasing Ataxin 2 mRNA and protein expression. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate Ataxin 2 associated diseases, disorders, and conditions. Such Ataxin 2 associated diseases include spinocerebellar ataxia type 2 (SCA2), amyotrophic sclerosis (ALS), and parkinsonism.



COMPOSITIONS FOR MODULATING ATAXIN 2 EXPRESSION

5 Statement of Government Support

This invention was made with government support under R21 NS081182 awarded by the National Institutes of Health. The government has certain rights in the invention.

Sequence Listing

10 The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0239WOSEQ_ST25.txt created March 19, 2015, which is 232 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

15 Field

Provided are compositions and methods for reducing expression of Ataxin 2 (ATXN2) mRNA and protein in an animal. Such methods are useful to treat, prevent, or ameliorate neurodegenerative diseases, including spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), and parkinsonism by inhibiting expression of Ataxin 2 by inhibiting expression of Ataxin 2 in an animal.

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Background

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurodegenerative disease characterized by progressive functional and cell loss of neurons in the cerebellum, brain stem and spinal cord. The cause of SCA2 is CAG expansion in the ATXN2 gene resulting in polyglutamine (polyQ) expansion in the ataxin-2 protein. Patients with SCA2 are characterized by progressive cerebellar ataxia, slow saccadic eye movements and other neurologic features such as neuropathy (Pulst, S.M. (ed.), *Genetics of Movement Disorders*. Elsevier, Inc., Amsterdam, 2003, pp. 19–34.). Moderate CAG expansion in the ATXN2 gene is also associated with parkinsonism or amyotrophic lateral sclerosis (ALS) indistinguishable from the idiopathic forms of these diseases (Kim *et al.*, *Arch. Neurol.*, 2007, 64: 1510–1518; Ross *et al.*, *Hum. Mol. Genet.*, 2011, 20: 3207–3212; Corrado *et al.*, *Hum. Genet.*, 2011, 130: 575–580; Elden *et al.*, *Nature*, 2010, 466: 1069–1075; Van Damme *et al.*, *Neurology*, 2011, 76: 2066–2072).

The pathogenic functions of polyQ disease proteins that occur with polyQ expansion may be attributed to the gain of toxicity associated with the development of intranuclear inclusion bodies or with soluble toxic oligomers (Lajoie *et al.*, *PLoS One*, 2011, 5: e15245). While SCA2 patient brains are characterized by loss of Purkinje cells, SCA2 Purkinje cells lack inclusion bodies indicating polyQ-expanded

ataxin-2 may cause toxicity that is unrelated to inclusion body formation (Huynh *et al.*, *Ann. Neurol.*, 1999, 45: 232–241). Functions gained in polyQ-expanded ataxin-2 may include anomalous accumulation in Golgi bodies (Huynh *et al.*, *Hum. Mol. Genet.*, 2003, 12: 1485–1496), gain-of-normal functions (Duvick *et al.*, *Neuron*, 2010, 67: 929–935) and sequestering of transcription factors (TFs) and glyceraldehyde- 3-phosphate dehydrogenase like for other polyQ proteins (Yamanaka *et al.*, *Methods Mol. Biol.*, 2010: 648, 215–229; Koshy *et al.*, *Hum. Mol. Genet.*, 1996, 5: 1311–1318; Burke *et al.*, *Nat. Med.*, 1996, 2: 347–350). Some normal functions of ataxin-2 have been characterized. Ataxin-2 is present in stress granules and P-bodies suggesting functions in sequestering mRNAs and protein translation regulation during stress (Nonhoff *et al.*, *Mol. Biol. Cell*, 2007, 18: 1385–1396). Ataxin-2 overexpression interfered with the P-body assembly, while underexpression interfered with stress granule assembly (Nonhoff *et al.*, *Mol. Biol. Cell*, 2007, 18: 1385–1396). Interactions with polyA-binding protein 1, the RNA splicing factor A2BP1/Fox1 and polyribosomes further support roles for ataxin-2 in RNA metabolism (Shibata *et al.*, *Hum. Mol. Genet.*, 2000, 9: 1303–1313; Ciosk *et al.*, *Development*, 2004, 131: 4831–4841; Satterfield *et al.*, *Hum. Mol. Genet.*, 2006, 15: 2523–2532). Ataxin-2 is a regulator of EGF receptor internalization and signaling by the way of its interactions with SRC kinase and the endocytic protein CIN85 (Nonis *et al.*, *Cell Signal.*, 2008, 20: 1725–1739). Ataxin-2 also interacts with the ALS-related protein TDP-43 in an RNA-dependent manner and familial and sporadic ALS associates with the occurrence of long normal CAG repeat expansion ATXN2 (Elden *et al.*, *Nature*, 2010, 466: 1069–1075; Van Damme *et al.*, *Neurology*, 2011, 76: 2066–2072).

Currently there is a lack of acceptable options for treating such neurodegenerative diseases. It is therefore an object herein to provide methods for the treatment of such diseases.

Summary

Provided herein are methods, compounds, and compositions for modulating expression of Ataxin 2 (ATXN2) mRNA and protein. In certain embodiments, compounds useful for modulating expression of Ataxin 2 mRNA and protein are antisense compounds. In certain embodiments, the antisense compounds are modified oligonucleotides.

In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, Ataxin 2 mRNA levels are reduced. In certain embodiments, Ataxin 2 protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such Ataxin 2 related diseases, disorders, and conditions are neurodegenerative diseases. In certain embodiments, such neurodegenerative diseases, disorders, and conditions include spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), and parkinsonism.

Such diseases, disorders, and conditions can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of neurodegenerative disorder include growing older, having a personal or family history, or genetic predisposition. Certain symptoms and outcomes associated with development of a neurodegenerative disorder include but are not limited to: ataxia, speech and swallowing difficulties, rigidity, tremors, ophthalmoplegia, saccadic slowing, peripheral neuropathy, atrophy, dystonia, chorea, and dementia.

In certain embodiments, methods of treatment include administering an Ataxin 2 antisense compound to an individual in need thereof. In certain embodiments, methods of treatment include administering an Ataxin 2 modified oligonucleotide to an individual in need thereof.

Detailed Description

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

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

Definitions

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

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

“2’-O-methoxyethyl” (also 2’-MOE and 2’-OCH₂CH₂-OCH₃ and MOE) refers to an O-methoxyethyl modification of the 2’ position of a furanose ring. A 2’-O-methoxyethyl modified sugar is a modified sugar.

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

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

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

“About” means within $\pm 7\%$ of a value. For example, if it is stated, “the compounds affected at least about 70% inhibition of Ataxin 2”, it is implied that the Ataxin 2 levels are inhibited within a range of 63% and 77%.

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

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

“Amelioration” refers to a lessening, slowing, stopping, or reversing of at least one indicator of the severity of a condition or disease. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

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

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

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

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

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

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

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

“Ataxin 2” means the mammalian gene Ataxin 2 (ATXN2), including the human gene Ataxin 2 (ATXN2). Human Ataxin 2 has been mapped to human chromosome 12q24.1.

“Ataxin 2 associated disease” means any disease associated with any Ataxin 2 nucleic acid or expression product thereof. Such diseases may include a neurodegenerative disease. Such neurodegenerative diseases may include spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), and parkinsonism.

“Ataxin 2 mRNA” means any messenger RNA expression product of a DNA sequence encoding Ataxin 2.

“Ataxin 2 nucleic acid” means any nucleic acid encoding Ataxin 2. For example, in certain embodiments, an Ataxin 2 nucleic acid includes a DNA sequence encoding Ataxin 2, an RNA sequence transcribed from DNA encoding Ataxin 2 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding Ataxin 2. “Ataxin 2 mRNA” means an mRNA encoding an Ataxin 2 protein.

“Ataxin 2 protein” means the polypeptide expression product of an Ataxin 2 nucleic acid.

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

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

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

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

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

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

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

“Chimeric antisense compound” means an antisense compound that has at least two chemically distinct regions, each position having a plurality of subunits.

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

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

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

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

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

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

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

“Effective amount” in the context of modulating an activity or of treating or preventing a condition means the administration of that amount of pharmaceutical agent to a subject in need of such modulation, treatment, or prophylaxis, either in a single dose or as part of a series, that is effective for modulation of that effect, or for treatment or prophylaxis or improvement of that condition. The effective amount may vary among individuals depending on the health and physical condition of the individual to be treated, the

taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

"Efficacy" means the ability to produce a desired effect.

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

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

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

15 "Gap-narrowed" means a chimeric antisense compound having a gap segment of 9 or fewer contiguous 2'-deoxyribonucleosides positioned between and immediately adjacent to 5' and 3' wing segments having from 1 to 6 nucleosides.

"Gap-widened" means a chimeric antisense compound having a gap segment of 12 or more contiguous 2'-deoxyribonucleosides positioned between and immediately adjacent to 5' and 3' wing segments having from 1 to 6 nucleosides.

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

25 "Identifying an animal having an Ataxin 2 associated disease" means identifying an animal having been diagnosed with an Ataxin 2 associated disease or predisposed to develop an Ataxin 2 associated disease. Individuals predisposed to develop an Ataxin 2 associated disease include those having one or more risk factors for developing an Ataxin 2 associated disease, including, growing older, having a personal or family history, or genetic predisposition of one or more Ataxin 2 associated diseases. Such identification may be accomplished by any method including evaluating an individual's medical history and standard clinical tests or assessments, such as genetic testing.

30 "Immediately adjacent" means there are no intervening elements between the immediately adjacent elements.

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

35 "Inhibiting Ataxin 2" means reducing the level or expression of an Ataxin 2 mRNA and/or protein. In certain embodiments, Ataxin 2 mRNA and/or protein levels are inhibited in the presence of an antisense

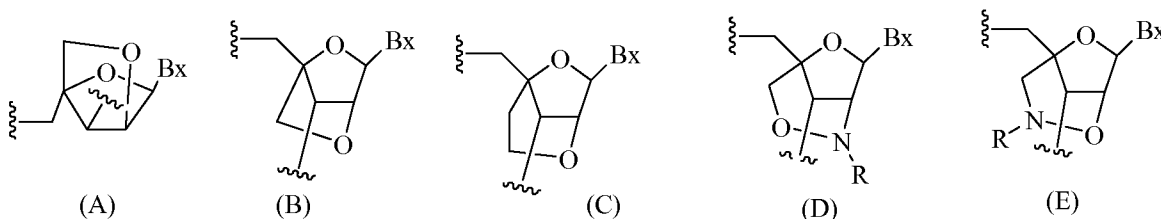
compound targeting Ataxin 2, including an antisense oligonucleotide targeting Ataxin 2, as compared to expression of Ataxin 2 mRNA and/or protein levels in the absence of an Ataxin 2 antisense compound, such as an antisense oligonucleotide.

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

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

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

“Locked nucleic acid” or “LNA” or “LNA nucleosides” means nucleic acid monomers having a bridge connecting two carbon atoms between the 4' and 2' position of the nucleoside sugar unit, thereby forming a bicyclic sugar. Examples of such bicyclic sugar include, but are not limited to A) α -L-Methyleneoxy (4'-CH₂-O-2') LNA, (B) β -D-Methyleneoxy (4'-CH₂-O-2') LNA, (C) Ethyleneoxy (4'-(CH₂)₂-O-2') LNA, (D) Aminooxy (4'-CH₂-O-N(R)-2') LNA and (E) Oxyamino (4'-CH₂-N(R)-O-2') LNA, as depicted below.



As used herein, LNA compounds include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the sugar wherein each of the bridges independently comprises 1 or from 2 to 4 linked groups independently selected from $-[C(R_1)(R_2)]_n-$, $-C(R_1)=C(R_2)-$, $-C(R_1)=N-$, $-C(=NR_1)-$, $-C(=O)-$, $-C(=S)-$, $-O-$, $-Si(R_1)_2-$, $-S(=O)_x-$ and $-N(R_1)-$; wherein: x is 0, 1, or 2; n is 1, 2, 3, or 4; each R_1 and R_2 is, independently, H, a protecting group, hydroxyl, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, a heterocycle radical, a substituted heterocycle radical, heteroaryl, substituted heteroaryl, C₅-C₇ alicyclic radical, substituted C₅-C₇ alicyclic radical, halogen, OJ₁, NJ₁J₂, SJ₁, N₃, COOJ₁, acyl (C(=O)-H), substituted acyl, CN, sulfonyl (S(=O)₂-J₁), or sulfoxyl (S(=O)-J₁); and each J₁ and J₂ is, independently, H, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C₁-C₁₂ aminoalkyl, substituted C₁-C₁₂ aminoalkyl or a protecting group.

Examples of 4'-2' bridging groups encompassed within the definition of LNA include, but are not limited to one of formulae: $-[C(R_1)(R_2)]_n-$, $-[C(R_1)(R_2)]_n-O-$, $-C(R_1R_2)-N(R_1)-O-$ or $-C(R_1R_2)-O-N(R_1)-$.

Furthermore, other bridging groups encompassed with the definition of LNA are 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R₁)-2' and 4'-CH₂-N(R₁)-O-2'- bridges, wherein each R₁ and R₂ is, independently, H, a protecting group or C₁-C₁₂ alkyl.

Also included within the definition of LNA according to the invention are LNAs in which the 2'-hydroxyl group of the ribosyl sugar ring is connected to the 4' carbon atom of the sugar ring, thereby forming a methyleneoxy (4'-CH₂-O-2') bridge to form the bicyclic sugar moiety. The bridge can also be a methylene (-CH₂-) group connecting the 2' oxygen atom and the 4' carbon atom, for which the term methyleneoxy (4'-CH₂-O-2') LNA is used. Furthermore; in the case of the bicyclic sugar moiety having an ethylene bridging group in this position, the term ethyleneoxy (4'-CH₂CH₂-O-2') LNA is used. α -L- methyleneoxy (4'-CH₂-O-2'), an isomer of methyleneoxy (4'-CH₂-O-2') LNA is also encompassed within the definition of LNA, as used herein.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

"Nucleobase complementarity" refers to a nucleobase that is capable of base pairing with another nucleobase. For example, in DNA, adenine (A) is complementary to thymine (T). For example, in RNA, adenine (A) is complementary to uracil (U). In certain embodiments, complementary nucleobase refers to a

nucleobase of an antisense compound that is capable of base pairing with a nucleobase of its target nucleic acid. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair.

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

“Nucleoside” means a nucleobase linked to a sugar.

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

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

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

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

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

“Parenteral administration” means administration through injection (*e.g.*, bolus injection) or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, *e.g.*, intrathecal or intracerebroventricular administration.

“Peptide” means a molecule formed by linking at least two amino acids by amide bonds. Without limitation, as used herein, peptide refers to polypeptides and proteins.

“Pharmaceutical agent” means a substance that provides a therapeutic benefit when administered to an individual. For example, in certain embodiments, an antisense oligonucleotide targeted to Ataxin 2 is a pharmaceutical agent.

“Pharmaceutical composition” means a mixture of substances suitable for administering to a subject. For example, a pharmaceutical composition may comprise an antisense oligonucleotide and a sterile aqueous solution.

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

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

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

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

“Prevent” or “preventing” refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to days, weeks to months, or indefinitely.

“Prodrug” means a therapeutic agent that is prepared in an inactive form that is converted to an active form (*i.e.*, drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions.

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

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

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

“Salts” mean a physiologically and pharmaceutically acceptable salts of antisense compounds, *i.e.*, salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

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

“Shortened” or “truncated” versions of antisense oligonucleotides taught herein have one, two or more nucleosides deleted.

“Side effects” means physiological responses attributable to a treatment other than desired effects. In certain embodiments, side effects include, without limitation, injection site reactions, liver function test

abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies.

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

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

“Slows progression” means decrease in the development of the disease.

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

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

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

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

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

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

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

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

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

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

“Treat” or “treating” or “treatment” refers administering a composition to effect an alteration or improvement of the disease or condition.

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

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

35 “Wing segment” means a plurality of nucleosides modified to impart to an oligonucleotide properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

Certain Embodiments

Certain embodiments provide methods, compounds, and compositions for inhibiting Ataxin 2 mRNA and protein expression. Certain embodiments provide methods, compounds, and composition for decreasing
5 Ataxin 2 mRNA and protein levels.

Certain embodiments provide antisense compounds targeted to an Ataxin 2 nucleic acid. In certain embodiments, the Ataxin 2 nucleic acid is the sequence set forth in GENBANK Accession No. NM_002973.3 (incorporated herein as SEQ ID NO: 1), the complement of GENBANK Accession No. NT_009775.17 truncated from nucleotides 2465000 to 2616000 (incorporated herein as SEQ ID NO: 2) and
10 GENBANK Accession No. BX410018.2 (incorporated herein as SEQ ID NO: 3).

Certain embodiments provide methods for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with Ataxin 2 in an individual in need thereof. Also contemplated are methods for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with Ataxin 2. Ataxin 2 associated diseases, disorders, and conditions
15 include neurodegenerative diseases. In certain embodiments, Ataxin 2 associated diseases include spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), and parkinsonism.

Certain embodiments provide compounds, comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20
20 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 11-165.

In certain embodiments the nucleobase sequence of the modified oligonucleotide is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID
25 NO: 3.

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

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

In certain emodiments, at least one modified internucleoside linkage is a phosphorothioate
30 internucleoside linkage.

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

In certain embodiments, at least one internucleoside linkage is a phosphodiester internucleoside linkage.

In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage and at least
35 one internucleoside linkage is a phosphodiester linkage.

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

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

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

5 In certain embodiments, at least one modified sugar is a bicyclic sugar.

In certain embodiments, the bicyclic sugar comprises a chemical link between the 2' and 4' position of the sugar 4'-CH₂-N(R)-O-2' bridge wherein R is, independently, H, C1-C12 alkyl, or a protecting group.

10 In certain embodiments, the bicyclic sugar comprises a 4'-CH₂-N(R)-O-2' bridge wherein R is, independently, H, C1-C12 alkyl, or a protecting group.

In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group.

In certain embodiments, the modified sugar comprises a 2'-O(CH₂)₂-OCH₃ group.

In certain embodiments, the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

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

a 3' wing segment consisting of 5 linked nucleosides;

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

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

20 Certain embodiments provide compositions comprising any compound described herein or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

Certain embodiments provide methods comprising administering to an animal any compound or composition described herein.

In certain embodiments, the animal is a human.

25 In certain embodiments, administering the compound prevents, treats, ameliorates, or slows progression of an Ataxin 2 associated disease, disorder or condition.

In certain embodiments, the Ataxin 2 disease, disorder or condition spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), and parkinsonism.

30 Certain embodiments provide use of any of the compounds or compositions of described herein for the manufacture of a medicament for treating a neurodegenerative disorder.

Antisense Compounds

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

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

In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 12 to 30 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 12 to 25 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 12 to 22 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 14 to 20 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 15 to 25 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 18 to 22 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 19 to 21 subunits in length. In certain embodiments, the antisense compound is 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 30, 18 to 50, 19 to 30, 19 to 50, or 20 to 30 linked subunits in length.

In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 12 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 13 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 14 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 15 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 16 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 17 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 18 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 19 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 20 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 21 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 22 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 23 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 24 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 25 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 26 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 27 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 28 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 29 subunits in length. In certain embodiments, an antisense compound targeted to an Ataxin 2 nucleic acid is 30 subunits in length. In certain embodiments, the antisense compound targeted to an Ataxin 2 nucleic acid is 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62,

63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In certain embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleosides.

In certain embodiments antisense oligonucleotides targeted to an Ataxin 2 nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to an Ataxin 2 nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

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

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

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

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

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to an Ataxin 2 nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

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

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE, and 2'-O-CH₃, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH₂)_n-O-2' bridge, where n=1 or n=2 and 4'-CH₂-O-CH₂-2'). In certain embodiments, wings may include several modified sugar moieties, including, for example 2'-MOE. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides and 2'-deoxynucleosides.

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

In certain embodiments, gapmers provided herein include, for example 20-mers having a motif of 5-10-5.

In certain embodiments, gapmers provided herein include, for example 19-mers having a motif of 5-9-5.

In certain embodiments, gapmers provided herein include, for example 18-mers having a motif of 5-8-5.

In certain embodiments, gapmers provided herein include, for example 18-mers having a motif of 4-8-6.

In certain embodiments, gapmers provided herein include, for example 18-mers having a motif of 6-8-4.

In certain embodiments, gapmers provided herein include, for example 18-mers having a motif of 5-7-6.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode Ataxin 2 include, without limitation, the following: GENBANK Accession No. NM_002973.3 (incorporated herein as SEQ ID NO: 1), the complement of GENBANK Accession No. NT_009775.17 truncated from nucleotides 2465000 to 2616000 (incorporated herein as SEQ ID NO: 2) and GENBANK Accession No. BX410018.2 (incorporated herein as SEQ ID NO: 3).

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

In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for Ataxin 2 can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

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

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain embodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more

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

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

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

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in Ataxin 2 mRNA levels are indicative of inhibition of Ataxin 2 expression. Reductions in levels of an Ataxin 2 protein are also indicative of inhibition of target mRNA expression. Phenotypic changes are indicative of inhibition of Ataxin 2 expression. Improvement in neurological function is indicative of inhibition of Ataxin 2 expression. Improved motor function and memory are indicative of inhibition of Ataxin 2 expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and an Ataxin 2 nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

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

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with an Ataxin 2 nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of

the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as an Ataxin 2 nucleic acid).

Non-complementary nucleobases between an antisense compound and an Ataxin 2 nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of an Ataxin 2 nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to an Ataxin 2 nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

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

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (*i.e.*, 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to an Ataxin 2 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, “fully complementary” means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be “fully complementary” to a target

sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as an Ataxin 2 nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as an Ataxin 2 nucleic acid, or specified portion thereof.

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

Identity

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

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

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

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

Modifications

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

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

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

Modified Internucleoside Linkages

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

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom.

Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to an Ataxin 2 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are interspersed throughout the antisense compound. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substituent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or C(R₁)(R₂) (R, R₁ and R₂ are each independently H, C₁-C₁₂ alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (*R* or *S*), 4'-S, 2'-F, 2'-OCH₃, 2'-OCH₂CH₃, 2'-OCH₂CH₂F and 2'-O(CH₂)₂OCH₃ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, OCF₃, OCH₂F, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(R_m)(R_n), O-CH₂-C(=O)-

$N(R_m)(R_n)$, and $O-CH_2-C(=O)-N(R_l)-(CH_2)_2-N(R_m)(R_n)$, where each R_l , R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-(CH_2)₂-O-2' (ENA); 4'-CH(CH_3)-O-2' and 4'-CH(CH_2OCH_3)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH_3)(CH_3)-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH_3)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH_3)-2' (see Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

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

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

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

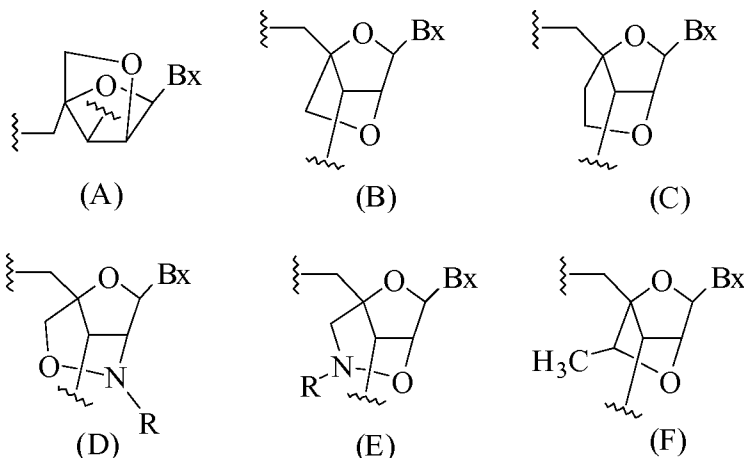
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, heterocycle radical,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

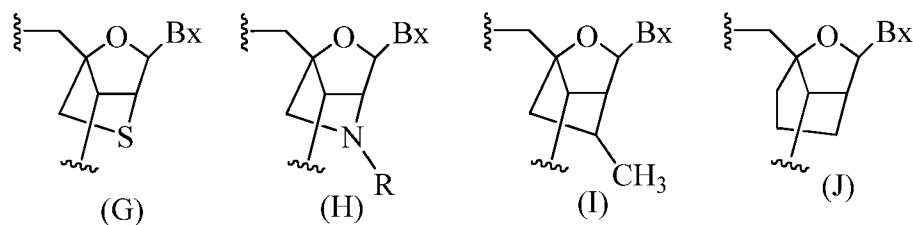
each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)$ -H), substituted acyl, a heterocycle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

In certain embodiments, the bridge of a bicyclic sugar moiety is $-[C(R_a)(R_b)]_n-$, $-[C(R_a)(R_b)]_n-O-$, $-C(R_aR_b)-N(R)-O-$ or $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is 4'- CH_2 -2', 4'-(CH_2)₂-2', 4'-(CH_2)₃-2', 4'- CH_2 -O-2', 4'-(CH_2)₂-O-2', 4'- CH_2 -O-N(R)-2' and 4'- CH_2 -N(R)-O-2' wherein each R is, independently, H, a protecting group or C_1 - C_{12} alkyl.

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

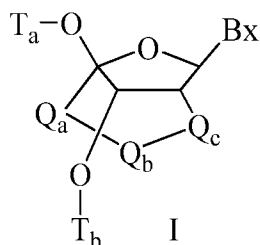
In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α -L-methyleneoxy (4'- CH_2 -O-2') BNA, (B) β -D-methyleneoxy (4'- CH_2 -O-2') BNA, (C) ethyleneoxy (4'-(CH_2)₂-O-2') BNA, (D) aminooxy (4'- CH_2 -O-N(R)-2') BNA, (E) oxyamino (4'- CH_2 -N(R)-O-2') BNA, and (F) methyl(methyleneoxy) (4'-CH(CH_3)-O-2') BNA, (G) methylene-thio (4'- CH_2 -S-2') BNA, (H) methylene-amino (4'- CH_2 -N(R)-2') BNA, (I) methyl carbocyclic (4'- CH_2 -CH(CH_3)-2') BNA, and (J) propylene carbocyclic (4'-(CH_2)₃-2') BNA as depicted below.





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

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



wherein:

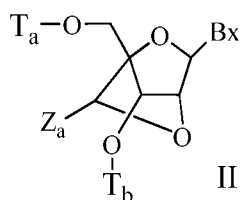
Bx is a heterocyclic base moiety;

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

R_c is C₁-C₁₂ alkyl or an amino protecting group; and

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

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



wherein:

20 Bx is a heterocyclic base moiety;

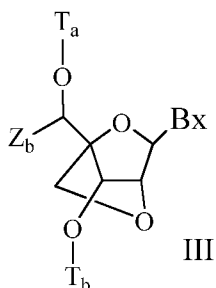
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

Z_a is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₁-C₆ alkyl, substituted C₂-C₆ alkenyl, substituted C₂-C₆ alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio.

25 In one embodiment, each of the substituted groups is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c, NJ_cJ_d, SJ_c, N₃, OC(=X)J_c, and

$\text{NJ}_e\text{C}(=\text{X})\text{NJ}_c\text{J}_d$, wherein each J_c , J_d and J_e is, independently, H, $\text{C}_1\text{-C}_6$ alkyl, or substituted $\text{C}_1\text{-C}_6$ alkyl and X is O or NJ_c .

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



5

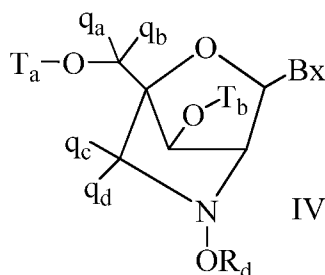
wherein:

Bx is a heterocyclic base moiety;

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

10 Zb is $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, substituted $\text{C}_1\text{-C}_6$ alkyl, substituted $\text{C}_2\text{-C}_6$ alkenyl, substituted $\text{C}_2\text{-C}_6$ alkynyl or substituted acyl ($\text{C}(=\text{O})\text{-}$).

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



15

wherein:

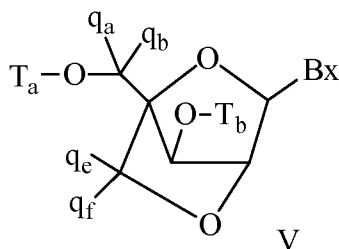
Bx is a heterocyclic base moiety;

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

20 Rd is $\text{C}_1\text{-C}_6$ alkyl, substituted $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, substituted $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl or substituted $\text{C}_2\text{-C}_6$ alkynyl;

each q_a , q_b , q_c and q_d is, independently, H, halogen, $\text{C}_1\text{-C}_6$ alkyl, substituted $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, substituted $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl or substituted $\text{C}_2\text{-C}_6$ alkynyl, $\text{C}_1\text{-C}_6$ alkoxy, substituted $\text{C}_1\text{-C}_6$ alkoxy, acyl, substituted acyl, $\text{C}_1\text{-C}_6$ aminoalkyl or substituted $\text{C}_1\text{-C}_6$ aminoalkyl;

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



wherein:

Bx is a heterocyclic base moiety;

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

qa, qb, qe and qf are each, independently, hydrogen, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k,

10 N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k or N(H)C(=S)NJ_jJ_k;

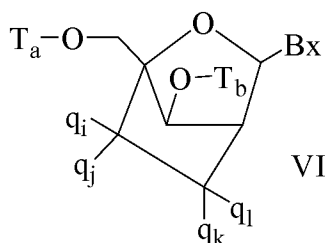
or qe and qf together are =C(q_g)(q_h);

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

The synthesis and preparation of the methyleneoxy (4'-CH₂-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid
15 recognition properties have been described (Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

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

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



wherein:

Bx is a heterocyclic base moiety;

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

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

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

One carbocyclic bicyclic nucleoside having a 4'-(CH_2)₃-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH₂-2' have been described (Freier *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4429-4443 and Alback *et al.*, *J. Org. Chem.*, 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava *et al.*, *J. Am. Chem. Soc.*, 2007, 129(26), 8362-8379).

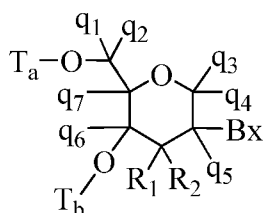
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

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

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

Chim. Acta, 1995, 78, 486-504; Altmann *et al.*, *Chimia*, 1996, 50, 168-176; Altmann *et al.*, *Biochem. Soc. Trans.*, 1996, 24, 630-637; and Altmann *et al.*, *Nucleosides Nucleotides*, 1997, 16, 917-926).

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



VII

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

Bx is a heterocyclic base moiety;

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

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

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

As used herein, “2'-modified” or “2'-substituted” refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring; and nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, -OCF₃, O-(CH₂)₂-O-CH₃, 2'-O(CH₂)₂SCH₃, O-(CH₂)₂-O-

$N(R_m)(R_n)$, or $O-CH_2-C(=O)-N(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. 2'-modified nucleosides may further comprise other modifications, for example at other positions of the sugar and/or at the nucleobase.

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

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

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

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

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

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

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

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

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

Compositions and Methods for Formulating Pharmaceutical Compositions

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

An antisense compound targeted to an Ataxin 2 nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to an Ataxin 2 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the

pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

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

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

Conjugated Antisense Compounds

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

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

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of Ataxin 2 nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commercial vendors (e.g. American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HepG2 cells, Hep3B cells, and primary hepatocytes.

In vitro testing of antisense oligonucleotides

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

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

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

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

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

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

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

RNA Isolation

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

Analysis of inhibition of target levels or expression

Inhibition of levels or expression of an Ataxin 2 nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis,

competitive polymerase chain reaction (PCR), or quantitative real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-
5 Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

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

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

Probes and primers are designed to hybridize to an Ataxin 2 nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as
25 PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of Ataxin 2 nucleic acids can be assessed by measuring Ataxin 2 protein levels. Protein levels of Ataxin 2 can be evaluated or quantitated in a variety of ways well known in the art, such as
30 immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or
35 polyclonal antibody generation methods well known in the art.

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of Ataxin 2 and produce phenotypic changes, such as, improved motor function and cognition. In certain embodiments, motor function is measured by walking initiation analysis, rotarod, grip strength, pole climb, open field performance, balance beam, hindpaw footprint testing in the animal.

Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, and subcutaneous. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from CNS tissue or CSF and changes in Ataxin 2 nucleic acid expression are measured.

Certain Indications

In certain embodiments, provided herein are methods, compounds, and compositions of treating an individual comprising administering one or more pharmaceutical compositions described herein. In certain embodiments, the individual has a neurodegenerative disease. In certain embodiments, the individual is at risk for developing a neurodegenerative disease, including, but not limited to, spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), and parkinsonism. In certain embodiments, the individual has been identified as having an Ataxin 2 associated disease. In certain embodiments, provided herein are methods for prophylactically reducing Ataxin 2 expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to an Ataxin 2 nucleic acid.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to an Ataxin 2 nucleic acid is accompanied by monitoring of Ataxin 2 levels in an individual, to determine an individual's response to administration of the antisense compound. An individual's response to administration of the antisense compound may be used by a physician to determine the amount and duration of therapeutic intervention.

In certain embodiments, administration of an antisense compound targeted to an Ataxin 2 nucleic acid results in reduction of Ataxin 2 expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to an Ataxin 2 nucleic acid results in improved motor function in an animal. In certain embodiments, administration of an Ataxin 2 antisense compound improves motor function by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 56, 57, 58, 59, 60, 61, 62,

63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to Ataxin 2 are used for the preparation of a medicament for treating a patient suffering or susceptible to a neurodegenerative disease including spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), and parkinsonism.

EXAMPLES

Non-limiting disclosure and incorporation by reference

While certain compounds, compositions, and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references recited in the present application is incorporated herein by reference in its entirety.

Example 1: Antisense inhibition of human ataxin 2 in HepG2 cells by MOE gapmers

Antisense oligonucleotides were designed targeting an ataxin 2 nucleic acid and were tested for their effects on ataxin 2 mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 4,500 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and ataxin 2 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3642 (forward sequence ACCAAAGAGTAGTTAATGGAGGTGTTC, designated herein as SEQ ID NO: 5; reverse sequence AGAAGGTGGGCGAGAGGAA, designated herein as SEQ ID NO: 6; probe sequence CTGGCCATCGCCTTGCCCA, designated herein as SEQ ID NO: 7) was used to measure mRNA levels. Ataxin 2 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of ataxin 2, relative to untreated control cells.

The chimeric antisense oligonucleotides in the Tables below were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most

nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human ataxin 2 mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_002973.3) or the human ataxin 2 genomic sequence, designated herein as SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_009775.17 truncated from nucleotides 2465000 to 2616000).

- 5 Some oligonucleotides do not target either SEQ ID NO: 1 or SEQ ID NO: 2, but instead target a variant gene sequence, SEQ ID NO: 3 (GENBANK Accession No. BX410018.2). 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 1

Inhibition of ataxin 2 mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
564118	606	625	CCGGCTCGCACGCCGGGCGG	57	2596	2615	11
564119	612	631	CATACACCGGCTCGCACGCC	63	2602	2621	12
564120	637	656	GGCTTCAGCGACATGGTGAG	78	2627	2646	13
564121	880	899	CGACCTCTGCCCAGGCCGGG	67	n/a	n/a	14
564122	935	954	TGCATAGATTCCATCAAAAG	90	47454	47473	15
564123	959	978	AAGTATATGAACCATCCTCA	67	47478	47497	16
564124	997	1016	TTCATTGTACTTCACATTT	85	48696	48715	17
564125	1084	1103	TCTGTACTTTTCTCATGTGC	88	49258	49277	18
564126	1090	1109	CTGGATTCTGTACTTTTCTC	89	49264	49283	19
564127	1123	1142	CTCTCCATTATTTCTTCACG	92	49297	49316	20
564128	1168	1187	TCTTTAAACTGTACCACAAC	86	49342	49361	21
564129	1210	1229	GAGTCAGTAAAAGCATCTCT	84	n/a	n/a	22
564130	1264	1283	CAGGGCTCCAGGTCCTTCTC	83	76401	76420	23
564131	1270	1289	GCATCCCAGGGCTCCAGGTC	86	76407	76426	24
564132	1363	1382	TCTTCATTATATCGAAACAT	84	80718	80737	25
564133	1477	1496	GCTAACTGGTTTGCCCTTGC	98	81637	81656	26
564134	1556	1575	GTATTTTCTTCCTCACTCC	82	81716	81735	27
564135	1562	1581	TGCTGTGTATTTTCTTCCT	89	81722	81741	28
564136	1748	1767	GAAATCTGAAGTGTGAGAAG	61	83359	83378	29
564137	1789	1808	CCTCCATTAACCTACTCTTG	90	83400	83419	30
564138	1795	1814	GGAACACCTCCATTAACCTAC	66	n/a	n/a	31
564139	1807	1826	GGCGATGGCCAGGGAACACC	95	85303	85322	32
564140	1844	1863	GTAGCGAGAAGGTGGGCGAG	88	85340	85359	33
564141	1862	1881	AGAGTTGGGACCTGACTGGT	84	85358	85377	34
564142	1868	1887	TGGAAGAGAGTTGGGACCTG	84	85364	85383	35
564143	1963	1982	GGAGCTGGAGAACCATGAGC	91	85459	85478	36
564144	1969	1988	GAGACAGGAGCTGGAGAACC	86	85465	85484	37
564145	2101	2120	TTGTGGGATACAAATTCTAG	56	88211	88230	38

564146	2185	2204	GGAACCCCACTGACCACTGA	70	n/a	n/a	39
564147	2401	2420	TCTTGAAGCCTGGAATCTTT	61	91671	91690	40
564148	2560	2579	AACCTAAAATCATTCTTAAA	21	n/a	n/a	41
564149	2596	2615	AGTTGATCCATAGATTGAGA	74	112905	112924	42
564150	2704	2723	CTGGTACAGTTGCTGCTGCT	91	113013	113032	43
564151	2710	2729	CTGCCACTGGTACAGTTGCT	85	113019	113038	44
564152	2899	2918	TTTGCATTGGGATTCAATGT	76	114859	114878	45
564153	2938	2957	GAAGGCTTTGGCTGAGAGAA	66	n/a	n/a	46
564154	2944	2963	GTAGTAGAAGGCTTTGGCTG	71	n/a	n/a	47
564155	2995	3014	TGACCCACCATAGATGGGCT	38	115850	115869	48
564156	3097	3116	GGTATTGGGTATAAAGGTTG	57	n/a	n/a	49
564157	3103	3122	GTCATAGGTATTGGGTATAA	76	116339	116358	50
564158	3331	3350	GGATGCTGAGACTGATAATG	54	n/a	n/a	51
564159	3337	3356	ACATGAGGATGCTGAGACTG	63	n/a	n/a	52
564160	3472	3491	AATTTGGGACATGCATACAT	23	n/a	n/a	53
564161	3490	3509	GTCTCCTTGTTGTATGGTAA	76	136963	136982	54
564162	3658	3677	TGAACAGGACTGGGTGCAGG	41	144433	144452	55
564163	3715	3734	GACTGCTGCTGTGGACTGGC	69	145447	145466	56
564164	3903	3922	CTGACTGTACATGAGCCTGA	50	147818	147837	57
564165	3909	3928	CCATTCCTGACTGTACATGA	69	147824	147843	58
564166	3927	3946	CAGTTGGATGAGAAGGAACC	58	147842	147861	59
564167	3933	3952	CATGGGCAGTTGGATGAGAA	29	147848	147867	60
564168	3971	3990	ACCGCCGGGTGGCTGTGTCG	40	147886	147905	61
564169	3993	4012	TTTGAGCGAGGGCGGCCTGG	19	147908	147927	62
564170	4005	4024	GCTGTAGTGCACCTTGAGCG	73	147920	147939	63
564171	4017	4036	AGACTGGAATGGGCTGTAGT	58	147932	147951	64
564172	4029	4048	GCGCTGTTGTGAGACTGGA	74	147944	147963	65
564173	4035	4054	GGAAATGCGCTGTTGTGAG	69	147950	147969	66
564174	4064	4083	GGCTTGTAAGGGTGCG	23	n/a	n/a	67
564175	4070	4089	GTGGTGGGCTTGTAAG	35	n/a	n/a	68
564176	4076	4095	CTGTTGGTGGTGGGCTTGTA	22	148827	148846	69
564177	4082	4101	CAACTGCTGTTGGTGGTGGG	39	148833	148852	70
564178	4088	4107	GCCTTACAAGCTGTTGGT	62	148839	148858	71
564179	4106	4125	TTCGGTTCCTCCAGGGCAGC	72	148857	148876	72
564180	4166	4185	TTCTAGTTTTCTGTGCTTCC	72	148917	148936	73
564181	4367	4386	AATAAATAACTTCCAGTTTC	59	149118	149137	74
564182	4429	4448	GAATCACTCTTGTTACTTCT	78	149180	149199	75
564183	4435	4454	CAGCAAGAATCACTCTTGTT	85	149186	149205	76
564184	4551	4570	TTTATAAATAAATCCGTC	4	149302	149321	77
564185	4593	4612	AAGTTGAACCACTGTAGACA	60	149344	149363	78
564186	n/a	n/a	ATCGGCCACCAACCGCGCGC	55	3683	3702	79
564187	n/a	n/a	CAAAGGGTTAATTAGGATCT	66	85057	85076	80
564188	n/a	n/a	CCCAAAGGGTTAATTAGGAT	94	85059	85078	81

564189	n/a	n/a	AGGACAGTCATTTGATTTGT	72	85166	85185	82
564190	n/a	n/a	CTTTGAGGACAGTCATTTGA	70	85171	85190	83
564191	n/a	n/a	CTGACAGAACAAATGATATG	17	85284	85303	84
564192	n/a	n/a	TATTGGGTATAAAGGCTTGA	31	116331	116350	85
564193	n/a	n/a	GGTATTGGGTATAAAGGCTT	78	116333	116352	86
564194	n/a	n/a	CTCTTTTACGCATACAGGCA	74	147789	147808	87
564195	n/a	n/a	AGGAAGGCCAACTGAGTCCT	70	148258	148277	88

Table 2

Inhibition of ataxin 2 mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
564158	3331	3350	GGATGCTGAGACTGATAATG	61	n/a	n/a	51
564196	70	89	GGTCAGACGGAAGCAGAACG	9	2060	2079	89
564197	218	237	CCACCTGGCTGCGGCGAAGC	12	2208	2227	90
564198	392	411	GCCGTTGCCGTTGCTACCAA	80	2382	2401	91
564199	616	635	GGCCCATACACCGGCTCGCA	79	2606	2625	92
564200	636	655	GCTTCAGCGACATGGTGAGG	81	2626	2645	93
564201	732	751	GGACATTGGCAGCCGCGGGC	83	2722	2741	94
564202	929	948	GATTCCATCAAAAGAAATCG	67	n/a	n/a	95
564203	969	988	CAACTGATGTAAGTATATGA	45	47488	47507	96
564204	1053	1072	CCAAATCACACTTCGGACTG	74	n/a	n/a	97
564205	1073	1092	CTCATGTGCGGCATCAAGTA	79	49247	49266	98
564206	1138	1157	CATTTGAACAAAATACTCTC	71	49312	49331	99
564207	1219	1238	CTGATAGCAGAGTCAGTAAA	72	76356	76375	100
564208	1521	1540	GGGCCACTCGAGCTTTGTAC	88	81681	81700	101
564209	1628	1647	AGGAATATATTTATTTTCCC	52	83239	83258	102
564210	1693	1712	CCCATACGCGGTGAATTCTG	91	83304	83323	103
564211	1713	1732	TGGAGCCCGATCCAGGCTGG	77	83324	83343	104
564212	1733	1752	AGAAGTGGATCTTGATGGCA	54	83344	83363	105
564213	1957	1976	GGAGAACCATGAGCAGAGGG	83	85453	85472	106
564214	2002	2021	GGCCCTTCTGAAGACATGCG	85	n/a	n/a	107
564215	2079	2098	CACTGGATATGGAACCCCTC	84	88189	88208	108
564216	2099	2118	GTGGGATACAAATTCTAGGC	94	88209	88228	109
564217	2177	2196	ACTGACCACTGATGACCACG	67	88287	88306	110
564218	2215	2234	CTGGGTCTATGAGTTTTAGG	67	91099	91118	111
564219	2291	2310	TGGAATAATACCAGCTTGGG	84	91175	91194	112
564220	2311	2330	GGCATGGCAACAGCTTCAGT	81	91195	91214	113
564221	2331	2350	TAGGAGATGCAGCTGGAATA	71	91215	91234	114
564222	2397	2416	GAAGCCTGGAATCTTTAGCC	69	n/a	n/a	115

564223	2426	2445	CCCTGCAGGAGAGTTCTGCC	75	91696	91715	116
564224	2582	2601	TTCAGAAGTAGAACTTGGCT	76	112891	112910	117
564225	2652	2671	CAATTTTGTCTTTGATCAAA	56	112961	112980	118
564226	2757	2776	TGTTACTAAGTATTGAAGGG	53	113066	113085	119
564227	2787	2806	AAGTGACCTCAGGTCCCCTC	83	113096	113115	120
564228	2883	2902	ATGTTGATTTCTAACTTGC	53	114843	114862	121
564229	3019	3038	GTATAAACTGGAGTTGGCTG	75	115874	115893	122
564230	3039	3058	GTGCAAAACAAACAGGCTGA	79	115894	115913	123
564231	3059	3078	GACTGGATACATCATATTTG	18	115914	115933	124
564232	3082	3101	GGTTGCACGCCTGGGCTCAC	74	n/a	n/a	125
564233	3102	3121	TCATAGGTATTGGGTATAAA	50	116338	116357	126
564234	3122	3141	TTGATTCACCTGGCATGGGCG	77	116358	116377	127
564235	3180	3199	GATGATGCTGGTCTTGCCGC	49	130944	130963	128
564236	3373	3392	ATCATTCTAGCATTACCCTG	61	131454	131473	129
564237	3408	3427	ATACTAAACCAGGCTGGGCG	71	131489	131508	130
564238	3464	3483	ACATGCATACATCGCATGCG	32	n/a	n/a	131
564239	3505	3524	TAGAAAGAAGGGCTTGTCTC	67	136978	136997	132
564240	3545	3564	CGCATACTGCTGAGCAAGGG	79	144320	144339	133
564241	3597	3616	TAGCTGAAGGCTGAGGGTGT	43	144372	144391	134
564242	3630	3649	CACCATGTTGGCTTTGCTGC	81	144405	144424	135
564243	3650	3669	ACTGGGTGCAGGATGACTTC	36	144425	144444	136
564244	3729	3748	CGTGGTAAATGGCTGACTGC	50	145461	145480	137
564245	3772	3791	TTGGAGGCAGGTGTCATGGA	36	145504	145523	138
564246	3938	3957	TGGCGCATGGGCAGTTGGAT	67	147853	147872	139
564247	3994	4013	CTTTGAGCGAGGGCGGCCTG	29	147909	147928	140
564248	4021	4040	GTCGAGACTGGAATGGGCTG	54	147936	147955	141
564249	4225	4244	ATTCCTATTGGATGTTACAA	65	148976	148995	142
564250	4252	4271	ATCTTCCACTGCAAGTGAAC	77	149003	149022	143
564251	4306	4325	TATGGAATTATGGAATAGCC	65	149057	149076	144
564252	4433	4452	GCAAGAATCACTCTTGTTAC	77	149184	149203	145
564253	4581	4600	TGTAGACAGTGATCACCTCA	77	149332	149351	146
564254	n/a	n/a	GGCCAAGGCCCACTTGTCTC	54	3485	3504	147
564255	n/a	n/a	CACTGCGGCCTCGAACAGCA	81	3709	3728	148
564263	n/a	n/a	AAATTCCTCATTTTCTTTTC	68	26924 27239	26943 27258	149
564264	n/a	n/a	GTTATAGTAATCTGTAATCA	71	36133 36239	36152 36258	150
564265	n/a	n/a	AGGATTGTAAAATGATACAG	47	65107 65148	65126 65167	151
564266	n/a	n/a	GTAGGATTGTAAAATGATAC	64	65109 65150	65128 65169	152
564267	n/a	n/a	TTATATATGTAAATTATATC	9	95228 95288	95247 95307	153

564268	n/a	n/a	AACCACTGATTTATACACTT	88	95260	95279	154
					95320	95339	
564269	n/a	n/a	TTAAAAACCACTGATTTATA	17	95265	95284	155
					95325	95344	
564270	n/a	n/a	ATATAGCACTCTGCTGTATT	83	99282	99301	156
					99340	99359	
564271	n/a	n/a	TACCAAGCTTGTGGCTTGGG	32	137342	137361	157
					137420	137439	
564272	n/a	n/a	TTATACCAAGCTTGTGGCTT	52	137345	137364	158
					137423	137442	

Table 3

Inhibition of ataxin 2 mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 3

ISIS No	SEQ ID NO: 3 Start Site	SEQ ID NO: 3 Stop Site	Sequence	% inhibition	SEQ ID NO
564256	311	330	CCTCGATGTTCCACAGGCGC	83	159
564257	715	734	GAGTTCACCTGCATCCAGGG	81	160
564258	736	755	TCCAGTTCCTCATTGGCTG	27	161
564259	771	790	GGTTCATCCATTAGATACG	52	162
564260	791	810	TTAAACGAAACATATCTTTG	10	163
564261	815	834	GCCCCTGCGCCATAATTTTT	3	164
564262	835	854	ATAAACTGCTTCAACGGTG	2	165

5 Example 2: Dose-dependent antisense inhibition of human ataxin 2 in HepG2 cells by MOE gapmers

Gapmers from Example 1 exhibiting significant *in vitro* inhibition of ataxin 2 mRNA were selected and tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.625 μ M, 1.250 μ M, 2.500 μ M, 5.000 μ M and 10.000 μ M concentrations of antisense oligonucleotide, as specified in the Table below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and ataxin 2 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3642 was used to measure mRNA levels. Ataxin 2 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of ataxin 2, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented. Ataxin 2 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 4
Dose response assay

ISIS No	0.625 μ M	1.250 μ M	2.500 μ M	5.000 μ M	10.000 μ M	IC ₅₀ (μ M)
564133	89	95	98	98	97	<0.6
564188	52	72	81	88	90	<0.6
564127	42	62	65	85	91	0.8
564150	39	63	74	86	91	0.8
564143	37	60	76	84	94	0.9
564122	25	53	69	85	88	1.3
564126	23	48	61	78	89	1.7
564144	12	35	53	71	85	2.4
564135	22	35	53	73	86	2.1
564125	33	44	64	78	85	1.5
564129	31	42	54	71	77	1.9
564216	50	67	82	86	94	<0.6
564210	33	48	72	80	94	1.3
564208	30	40	67	75	87	1.6
564268	35	52	69	81	85	1.2

Example 3: Antisense inhibition of human ataxin 2 in a SCA2 BAC mouse model

Gapmers from Example 1 exhibiting significant *in vitro* inhibition of ataxin 2 mRNA were selected and tested in vivo in a SCA2[Q22]-BAC mouse model. This mouse model was created in the Pulst laboratory (University of Utah, Salt Lake City), using mice of FVB/B6 hybrid background, for the study of spinocerebella ataxia type 2 (SCA2). These mice possess the entire 176 kb human ATXN2 gene region, including the 16 kb upstream sequence and the 2.5 kb downstream sequence.

Treatment

Groups of 3 mice each were administered normal saline (0.9%) or antisense oligonucleotide via intracerebroventricular injections. Five to seven week old mice were individually infused with a mixture of oxygen and 3% isoflurane for 3-4 minutes to cause sedation. The hair on the scalp was then removed with a shearing tool. The mouse was placed in a stereotaxic instrument (Stoelting Just for Mouse). The scalp was cleaned, first with an iodine scrub, and then with 70% ethanol. An incision was made with a #10 scalpel blade from the region just posterior to the place between the

eyes to the region 1.5 cm behind. The periosteum was removed with a sterile cotton swab. A Hamilton syringe with a 26-gauge needle was placed in the needle holder of the stereotaxic instrument and filled up to the 10 μ L mark with either normal saline (0.9%) or antisense oligonucleotide (250 μ g) in saline (0.9%) solution. The needle was positioned on the bregma on the skull, and then positioned 1 mm to the right and 0.46 mm posterior. The tip of the needle was then inserted just through the skull and then positioned 2.5 mm down into the right lateral ventricle. The plunger of the syringe was then depressed to deliver the desired volume of 5-7 μ L. After a wait of 4 minutes to allow ventricular pressure to equalize, the needle was removed and the scalp was sutured. The incision was then treated with povidone solution and the mouse returned to its cage on its back for recovery. The mice were monitored daily.

RNA analysis

After 7 days, the mice were placed in isoflurane until they were no longer breathing. The brain was then extracted. Three portions of the brain were collected in coronal sections, including one 3 mm section for RNA analysis. RNA was isolated from 30 mg tissue using the RNeasy kit (Qiagen). cDNA was generated using the QuantiTect Reverse Transcription kit (Qiagen). Real-time PCR (qPCR) was conducted by the SYBR Green method with standard curves on the iCycler (Bio-Rad) in 96-well plates in quadruplicate. Reactions were of 20 μ L, consisting of 15 ng cDNA, 2 μ L of each primer (0.3 μ M final), and 10 μ L SYBR Green Master Mix (Bio-Rad). Cycling parameters included a 95⁰ denaturation step for 10 seconds, incubation at the annealing temperature for 20 seconds, and a second incubation for 40 seconds at 72⁰. Each plate included a standard curve using cerebellar RNA prepared from multiple pGL2-5A3 transgenic mice. Single amplicons were verified by denaturation analysis and gel electrophoresis.

The results from the RNA analysis for mouse and human ataxin 2 are presented in the Table below. As indicated, some of the ISIS oligonucleotides decreased human ataxin 2 mRNA in the brains of the mice.

Table 5
Percent inhibition of ataxin 2 mRNA compared to the saline (0.9%) control in
SCA[Q22]-BAC mice

ISIS No	Human ataxin 2	Mouse ataxin 2
564122	10	15
564127	46	65
564133	60	62
564150	21	53

564188	9	23
564216	21	55

Example 4: Antisense inhibition of human ataxin 2 in an ATXN2-Q127 mouse model

Gapmers from Example 1 exhibiting significant *in vitro* inhibition of ataxin 2 mRNA were selected and tested in vivo in an ATXN2-Q127 mouse model. This mouse model (Hansen, S.T. et al., Human. Molecular Genetics 2012. 1-13) expresses the full-length-mutant ATXN2^{Q127} complementary DNA under the regulation of the Purkinje cell protein-2 (Pcp2) promoter. This model shows an early-onset progressive motor impairment phenotype accompanied by the formation of diffuse cytoplasmic aggregates in cerebellar Purkinje cells.

Treatment

Groups of 3 mice each were administered normal saline (0.9%) or antisense oligonucleotide via intracerebroventricular injections. Five to seven week old mice were individually infused with a mixture of oxygen and 3% isoflurane for 3-4 minutes to cause sedation. The hair on the scalp was then removed with a shearing tool. The mouse was placed in a stereotaxic instrument (Stoelting Just for Mouse). The scalp was cleaned, first with an iodine scrub, and then with 70% ethanol. An incision was made with a #10 scalpel blade from the region just posterior to the place between the eyes to the region 1.5 cm behind. The periosteum was removed with a sterile cotton swab. A Hamilton syringe with a 26-gauge needle was placed in the needle holder of the stereotaxic instrument and filled up to the 10 μ L mark with either normal saline (0.9%) or antisense oligonucleotide (250 μ g) in saline (0.9%) solution. The needle was positioned on the bregma on the skull, and then positioned 1 mm to the right and 0.46 mm posterior. The tip of the needle was then inserted just through the skull and then positioned 2.5 mm down into the right lateral ventricle. The plunger of the syringe was then depressed to deliver the desired volume of 5-7 μ L. After a wait of 4 minutes to allow ventricular pressure to equalize, the needle was removed and the scalp was sutured. The incision was then treated with povidone solution and the mouse returned to its cage on its back for recovery. The mice were monitored daily.

RNA analysis

After 7 days, the mice were placed in isoflurane until they were no longer breathing. The brain was then extracted. Three portions of the brain were collected in coronal sections, including one 3 mm section for RNA analysis. RNA was isolated from 30 mg tissue using the RNeasy kit (Qiagen). cDNA was generated using the QuantiTect Reverse Transcription kit (Qiagen). Real-

time PCR (qPCR) was conducted by the SYBR Green method with standard curves on the iCycler (Bio-Rad) in 96-well plates in quadruplicate. Reactions were of 20 μ L, consisting of 15 ng cDNA, 2 μ L of each primer (0.3 μ M final), and 10 μ L SYBR Green Master Mix (Bio-Rad). Cycling parameters included a 95⁰ denaturation step for 10 seconds, incubation at the annealing temperature for 20 seconds, and a second incubation for 40 seconds at 72⁰. Each plate included a standard curve using cerebellar RNA prepared from multiple pGL2-5A3 transgenic mice. Single amplicons were verified by denaturation analysis and gel electrophoresis. All mRNA levels were normalized to the housekeeping gene, actin.

The results from the RNA analysis for mouse and human ataxin 2 are presented in the Table below. As indicated, some of the ISIS oligonucleotides decreased human ataxin 2 mRNA in the brains of the mice.

qPCR analysis of the marker for microgliosis, AIF/Iba1, to measure inflammation, was also performed. The results are presented in the Table below.

Table 6
Percent inhibition of ataxin 2 mRNA compared to the saline (0.9%) control in ATXN2-Q127 mice

ISIS No	Human	Mouse
564133	64	52
564127	62	49
564216	46	40
564210	39	48

Table 7
Percent Iba1 mRNA level increase compared to the saline (0.9%) control in ATXN2-Q127 mice

ISIS No	Iba1
564133	9
564127	49
564216	16
564210	96

Example 4: Dose-dependent antisense inhibition of human ataxin 2 in an ATXN2-Q127 mouse model

ISIS 564133 was tested in different doses in the ATXN2-Q127 mouse model.

Treatment

Groups of 3 mice each were administered normal saline (0.9%) or ISIS 564133 via intracerebroventricular injections dosed at 50 µg, 100 µg, 200 µg, 250 µg, or 300 µg. The mice were administered in the same manner as described in the studies above and monitored daily.

5 *RNA analysis*

After 7 days, the mice were placed in isoflurane until they were no longer breathing. The brain was then extracted. Three portions of the brain were collected in coronal sections, including one 3 mm section for RNA analysis, as described above. All mRNA levels were normalized to the housekeeping gene, actin.

10 The results from the RNA analysis for mouse and human ataxin 2 are presented in the Table below.

Table 8
Percent inhibition of ataxin 2 mRNA compared to the saline (0.9%) control in ATXN2-Q127 mice

Dose (µg)	Human ataxin 2	Mouse ataxin 2
50	60	47
100	84	35
200	85	67
250	79	62
300	73	41

15

Example 5: Time-dependent antisense inhibition of human ataxin 2 in an ATXN2-Q127 mouse model

ISIS 564133 was administered and mRNA level reduction was tested in different time points in the ATXN2-Q127 mouse model.

20 *Treatment*

Groups of 3 mice each were administered normal saline (0.9%) or ISIS 564133 via intracerebroventricular injections dosed at 200 µg. The mice were administered in the same manner as described in the studies above and monitored daily.

RNA analysis

25 After 9 days, 18 days, 27 days, and 84 days, groups of mice were placed in isoflurane until they were no longer breathing. The brain was then extracted. Three portions of the brain were

collected in coronal sections, including one 3 mm section for RNA analysis, as described above. All mRNA levels were normalized to the housekeeping gene, actin.

The results from the RNA analysis for human ataxin 2 are presented in the Table below. Western analysis of the corresponding protein samples was performed and confirmed the qPCR results.

Table 9
Ataxin 2 mRNA levels in ATXN2-Q127 mice

Time Point	ATXN2 expression relative to actin
saline (0.9%) control	8.4
9 days	2.9
18 days	0.9
27 days	1.4
84 days	2.7

Immunohistochemical staining of cerebellar Purkinje cells on day 7 was performed using rabbit anti-oligonucleotide antibody generated in-house. The results demonstrated that ISIS oligonucleotide localized in cerebellar Purkinje cells of ATXN-Q127 mice.

Example 6: Effect of antisense inhibition of human ataxin 2 in an ATXN2-Q127 mouse model

ISIS oligonucleotide was administered in the ATXN2-Q127 mouse model and wild-type mice. On day 3, motor performance was evaluated using the rotarod test.

Groups of ATXN2-Q127 mice were administered normal saline (0.9%) or ISIS 564133 at 50 µg, 100 µg, or 200 µg via intracerebroventricular injections in the same manner as described in the studies above. Groups of wild-type mice were administered normal saline (0.9%) or ISIS oligonucleotide at 200 µg via intracerebroventricular injections dosed in the same manner as described in the studies above. Groups of ATXN2-Q127 mice were administered normal saline (0.9%) or ISIS 546127 or ISIS 564216 at 200 µg via intracerebroventricular injections dosed in the same manner as described in the studies above. After 6 weeks, the mice were subjected to the rotarod test.

Rotarod assay

The accelerating rotarod assay was performed on the Rotamex rotarod. Rotarod testing was conducted over five days. On the first day, mice are acclimated to the technician by handling the mice. On the second day mice are introduced to the rotarod in a 4 minutes paradigm including 2 minutes at a constant speed of 10 RPM, then 2 minutes at a speed ranging from 10 to 30 RPM.

- 5 Testing on days 3-5 were identical, where mice are placed on the rotarod at a speed of 0 RPM, then the rotarod was accelerated to 40 RPM over 6 minutes. This is done twice per day and a mean value of “latency to fall” per day was recorded, in seconds. Latency to fall is defined as the amount of time before the animal falls from the rotarod. It is recorded automatically, when the mouse no longer interrupts infrared beams directed above the rotarod. The time to first passive rotation (when mice
- 10 stop walking and hold on and revolve with the rod) is also automatically recorded, and generally reflects the latency to fall time. The study consisted of three consecutive trials of 5 minutes each with a 20 minute rest period between trials. On days 3-5, the mice were allowed to rest for 1.5-2 hrs between the two replicate tests conducted on each of those days.

- The results from the rotarod test are presented in the Table below. As shown in the Table
- 15 below, treatment with ASO improves rotarod performance by up to about 20%.

Table 10
Rotarod performance test in ATXN2-Q127 mice

Strain of mice	Number of mice	Treatment	Latency to fall (seconds)
WT	10	saline (0.9%) control	199
	10	ISIS 564133 (200 µg)	189
ATXN-Q127	8	saline (0.9%) control	127
	15	ISIS 564133 (50 µg)	149
	16	ISIS 564133 (100 µg)	141
	9	ISIS 564133 (200 µg)	100
ATXN-Q127	15	saline (0.9%) control	130
	13	ISIS 564127 (200 µg)	150
	15	ISIS 564216 (200 µg)	156

Example 7: Effect of antisense inhibition of human ataxin 2 in an ATXN2-Q127 mouse model

ISIS oligonucleotide was administered in the ATXN2-Q127 mouse model and wild-type mice. Cerebellar expression of ataxin 2, as well as several Purkinje cell (PC) genes, was assessed.

Groups of ATXN2-Q127 mice were administered normal saline (0.9%) or ISIS 564133 at 200 µg via intracerebroventricular injections dosed in the same manner as described in the studies above. Groups of wild-type mice were administered normal saline (0.9%) or ISIS 564133 at 200 µg via intracerebroventricular injections dosed in the same manner as described in the studies above. After 5 weeks, the mice were euthanized and cerebellar expression of various gene mRNA levels was assessed.

RNA analysis

Groups of mice were placed in isoflurane until they were no longer breathing. The brain was then extracted. Three portions of the brain were collected in coronal sections, including one 3 mm section for RNA analysis, as described above. All mRNA levels were normalized to the housekeeping gene, actin. RNA levels of human ataxin 2, murine ataxin 2, Pcp2, Calb1, Rgs8, and Fam107b were measured. Transcription changes in several of these PC-specific genes have been demonstrated to progressively decrease in models of SCA2 (Hansen, S.T. et al., Hum. Mol. Genet. 2013. 22: 271-283).

The results from the RNA analysis are presented in the Table below and demonstrate that treatment with ISIS oligonucleotides targeting ataxin 2 increased the expression levels of all the PC-specific genes compared to the transgenic control group.

Table 11
PC-specific mRNA levels in ATXN2-Q127 mice

	WT	ATXN-Q127	
	saline (0.9%) control	saline (0.9%) control	ISIS 564133 (200 µg)
human ataxin 2	0.21	3.57	1.31
murine ataxin 2	0.79	0.84	0.6
Pcp2	0.77	0.36	0.48
Rgs8	1.45	0.25	0.35
Calb1	1.14	0.5	0.71
Fam107b	1.41	0.7	0.9

Example 8: Effect of antisense inhibition of human ataxin 2 in an ATXN2-Q127 mouse model

ISIS oligonucleotide was administered in the ATXN2-Q127 mouse model and wild-type mice. Motor performance was evaluated using the rotarod test.

Groups of ATXN2-Q127 mice (7.5 weeks of age) were administered normal saline (0.9%) or ISIS 546127 or ISIS 564216 at 200 µg via intracerebroventricular injections dosed in the same manner as described in the studies above. After 5 weeks and 9 weeks, the mice were subjected to the rotarod test.

5 *Rotarod assay*

The accelerating rotarod assay was performed on the Rotamex rotarod. Rotarod testing was conducted over five days. On the first day, mice are acclimated to the technician by handling the mice. On the second day mice are introduced to the rotarod in a 4 minutes paradigm including 2 minutes at a constant speed of 10 RPM, then 2 minutes at a speed ranging from 10 to 30 RPM.

10 Testing on days 3-5 were identical, where mice are placed on the rotarod at a speed of 0 RPM, then the rotarod was accelerated to 40 RPM over 6 minutes. This is done twice per day and a mean value of “latency to fall” per day was recorded, in seconds. Latency to fall is defined as the amount of time before the animal falls from the rotarod. It is recorded automatically, when the mouse no longer interrupts infrared beams directed above the rotarod. The time to first passive rotation (when mice
15 stop walking and hold on and revolve with the rod) is also automatically recorded, and generally reflects the latency to fall time. The study consisted of three consecutive trials of 5 minutes each with a 20 minute rest period between trials. On days 3-5, the mice were allowed to rest for 1.5-2 hrs between the two replicate tests conducted on each of those days.

The results from the rotarod test are presented in the Table below. As shown in the Table
20 below, treatment with ASO improves rotarod performance by up to about 20% on week 5 and about 27% on week 9.

Table 12
Rotarod performance test in ATXN2-Q127 mice.
(mean latency to fall, in seconds)

Weeks after injection		ISIS 564127	ISIS 564216	Saline control
Week 5	DAY 3	137	145	123
	DAY 4	140	141	119
	DAY 5	155	154	131
Week 9	DAY 3	131	149	104
	DAY 4	125	139	104
	DAY 5	134	139	112

Example 9: Effect of antisense inhibition of human ataxin 2 in an ATXN2-Q127 mouse model

ISIS oligonucleotide was administered in the ATXN2-Q127 mouse model. Motor performance was evaluated using the rotarod test.

Seven week old ATXN2-Q127 mice were subjected to the rotarod test, then divided into two groups of 30 mice each, such that average rotarod performance, average weights, and sex composition were equal across both groups. At 8 weeks of age, one group of mice received normal saline via intracerebroventricular (ICV) injection and one group received ISIS 564216 at 210 µg via ICV injection, dosed in the same manner as described in the studies above. Five weeks later (13 weeks of age), the mice were again subjected to the rotarod test. Six weeks post injection (14 weeks of age), the mice received a second ICV injection, identical to the injection received at 8 weeks of age. Five weeks later (19 weeks of age, 11 weeks after the first ICV injection), the mice were subjected to a third rotarod test.

Rotarod test

The accelerating rotarod test was performed on the Rotamex rotarod. Rotarod testing was conducted over five days. On the first day, mice were acclimated to the technician by being handled by the technician three times, 5 minutes each time. On the second day, mice were introduced to the rotarod three times, 10 minutes each time at a speed ranging from 0 to 10 RPM. On each of days 3-5, mice were placed on the rotarod at a speed of 0 RPM, then the rotarod was accelerated to 40 RPM over 6 minutes, and this was done for each mouse three times. The three total trials per day were used to calculate a mean value of "latency to fall" per day, in seconds. Latency to fall is defined as the amount of time before the animal falls from the rotarod. It was recorded automatically, when the mouse no longer interrupted infrared beams directed above the rotarod. The time to first passive rotation (when mice stop walking and hold on and revolve with the rod) is also automatically recorded, and generally reflects the latency to fall time.

The results from the rotarod test are presented as the average for each treatment group in the Table below. As shown in the Table below, treatment with ASO improved rotarod performance.

Table 13
Rotarod performance test in ATXN2-Q127 mice

Treatment	Weeks after 1 st injection	Weeks after 2 nd injection	Testing day	Latency to fall (s)
Saline	5	n/a	3	218.5
			4	240.9
			5	236.5
Isis No. 564216	5	n/a	3	240.6
			4	257.9
			5	259.6
Saline	11	5	3	216.2
			4	198.7
			5	212.1
Isis No. 564216	11	5	3	194.4
			4	226.0
			5	242.8

CLAIMS:

What is claimed is:

1. A single-stranded modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising a portion of at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, or at least 20 contiguous nucleobases complementary to an equal length portion of nucleobases 1053-1109 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1 or SEQ ID NO: 2, wherein the modified oligonucleotide comprises:

- a gap segment consisting of linked deoxynucleosides;
- a 5' wing segment consisting of linked nucleosides; and
- a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar moiety or each wing segment comprises nucleosides comprising a modified sugar moiety and nucleosides comprising an unmodified sugar moiety, and wherein the single-stranded modified oligonucleotide reduces Ataxin-2 mRNA and/or protein expression.

2. A single-stranded modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or 20 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 18, 19 or 98, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1 or SEQ ID NO: 2, wherein the modified oligonucleotide comprises:

- a gap segment consisting of linked deoxynucleosides;
- a 5' wing segment consisting of linked nucleosides; and
- a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar moiety, or each wing segment comprises nucleosides comprising a modified sugar moiety and nucleosides comprising an unmodified sugar moiety, and wherein the single-stranded modified oligonucleotide reduces Ataxin-2 mRNA and/or protein expression.

3. The single-stranded modified oligonucleotide of claim 1 or 2, wherein the nucleobase sequence of the modified oligonucleotide is at least 95%, or 100% complementary to SEQ ID NO: 1 or

SEQ ID NO: 2.

4. The single-stranded modified oligonucleotide of any one of claims 1-3, wherein at least one internucleoside linkage is a modified internucleoside linkage; optionally wherein:
 - a) at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage; or
 - b) each modified internucleoside linkage is a phosphorothioate internucleoside linkage.
5. The single-stranded modified oligonucleotide of any one of claims 1-4, wherein:
 - a) at least one internucleoside linkage is a phosphodiester internucleoside linkage; and/or
 - b) at least one internucleoside linkage is a phosphorothioate linkage and at least one internucleoside linkage is a phosphodiester linkage.
6. The single-stranded modified oligonucleotide of any one of claims 1-5, wherein at least one nucleoside comprises a modified nucleobase; optionally wherein the modified nucleobase is a 5-methylcytosine.
7. The single-stranded modified oligonucleotide of any one of claims 1-6, wherein each nucleoside of each wing segment comprises a modified sugar moiety.
8. The single-stranded modified oligonucleotide of any one of claims 1-7, wherein at least one modified sugar moiety is a bicyclic sugar moiety; optionally wherein the bicyclic sugar moiety comprises a 4'-CH(R)-O-2' bridge wherein R is, independently, H, C₁-C₄ alkyl, or a protecting group; further optionally wherein:
 - a) R is methyl; or
 - b) R is H.
9. The single-stranded modified oligonucleotide of any one of claims 1-7, wherein at least one modified sugar moiety comprises a 2'-O-methoxyethyl group or a 2'-O-CH₃ group.
10. The single-stranded modified oligonucleotide of any one of claims 1-9, wherein the modified oligonucleotide comprises:
 - a gap segment consisting of 10 linked deoxynucleosides;
 - a 5' wing segment consisting of 5 linked nucleosides; and
 - a 3' wing segment consisting of 5 linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar moiety.

11. The single-stranded modified oligonucleotide of any one of claims 1-10, wherein the modified oligonucleotide consists of 20 linked nucleosides.
12. The single-stranded modified oligonucleotide of any one of claims 1-11, wherein the modified oligonucleotide comprises a conjugate group.
13. A single-stranded modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 15 consecutive nucleobases of SEQ ID NO: 18, wherein the nucleobase sequence of the modified oligonucleotide is 100% complementary to SEQ ID NO: 1 or SEQ ID NO: 2, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar moiety, wherein the at least one modified sugar moiety comprises a 2'-O-methoxyethyl group, wherein at least one nucleoside comprises a modified nucleobase that is a 5-methylcytosine, wherein at least one internucleoside linkage is a phosphorothioate linkage and at least one internucleoside linkage is a phosphodiester linkage, wherein the modified oligonucleotide comprises:
 - a gap segment consisting of linked deoxynucleosides;
 - a 5' wing segment consisting of linked nucleosides; and
 - a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a modified sugar moiety, and wherein the single-stranded modified oligonucleotide reduces Ataxin-2 mRNA and/or protein expression.
14. A single-stranded modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 19 consecutive nucleobases of SEQ ID NO: 19, wherein the nucleobase sequence of the modified oligonucleotide is 100% complementary to SEQ ID NO: 1 or SEQ ID NO: 2, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar moiety, wherein the at least one modified sugar moiety comprises a 2'-O-methoxyethyl group, wherein at least one nucleoside comprises a modified nucleobase that is a 5-methylcytosine, wherein at least one internucleoside linkage is a phosphorothioate linkage and at least one internucleoside linkage is a phosphodiester linkage, wherein the modified oligonucleotide comprises:
 - a gap segment consisting of linked deoxynucleosides;
 - a 5' wing segment consisting of linked nucleosides; and
 - a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a modified sugar moiety, and wherein the single-stranded modified oligonucleotide reduces Ataxin-2 mRNA and/or protein expression.

15. A composition comprising the single-stranded modified oligonucleotide of any one of claims 1-14 and at least one of a pharmaceutically acceptable carrier or diluent.
16. The composition of claim 15 wherein the pharmaceutically acceptable diluent is phosphate-buffered saline (PBS); and/or wherein the modified oligonucleotide is a sodium salt or a potassium salt.

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