



(51) International Patent Classification:

C12N 15/11 (2006.01) C07H 21/04 (2006.01)
A61K 48/00 (2006.01)

(21) International Application Number:

PCT/US2015/026218

(22) International Filing Date:

16 April 2015 (16.04.2015)

(25) Filing Language:

English

(26) Publication Language:

English

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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))



WO 2016/167780 A1

(54) Title: COMPOSITIONS FOR MODULATING EXPRESSION OF C9ORF72 ANTISENSE TRANSCRIPT

(57) Abstract: Disclosed herein are compositions and methods for modulating expression of C9ORF72 antisense transcript in a cell or animal with C9ORF72 antisense transcript specific inhibitors. Such methods are useful to treat, prevent, or ameliorate neurodegenerative diseases in an individual in need thereof. Such C9ORF72 antisense transcript specific inhibitors include antisense compounds.

COMPOSITIONS FOR MODULATING EXPRESSION OF C9ORF72 ANTISENSE TRANSCRIPT

5 **Sequence Listing**

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0261USLSEQ_ST25.txt created April 15, 2015, which is 84 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

10

Field

Provided are compositions and methods for modulating expression of C9ORF72 antisense transcript in an animal. Such compositions and methods are useful to treat, prevent, ameliorate, or slow progression of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS),
15 frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, and olivopontocerebellar degeneration (OPCD).

Background

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized
20 clinically by progressive paralysis leading to death from respiratory failure, typically within two to three years of symptom onset (Rowland and Shneider, N. Engl. J. Med., 2001, 344, 1688-1700). ALS is the third most common neurodegenerative disease in the Western world (Hirtz et al., Neurology, 2007, 68, 326-337), and there are currently no effective therapies. Approximately 10% of cases are familial in nature, whereas the bulk of patients diagnosed with the disease are classified
25 as sporadic as they appear to occur randomly throughout the population (Chio et al., Neurology, 2008, 70, 533-537). There is growing recognition, based on clinical, genetic, and epidemiological data, that ALS and frontotemporal dementia (FTD) represent an overlapping continuum of disease, characterized pathologically by the presence of TDP-43 positive inclusions throughout the central nervous system (Lillo and Hodges, J. Clin. Neurosci., 2009, 16, 1131-1135; Neumann et al.,
30 Science, 2006, 314, 130-133).

To date, a number of genes have been discovered as causative for classical familial ALS, for example, SOD1, TARDBP, FUS, OPTN, and VCP (Johnson et al., Neuron, 2010, 68, 857-864;

Kwiatkowski et al., *Science*, 2009, 323, 1205-1208; Maruyama et al., *Nature*, 2010, 465, 223-226; Rosen et al., *Nature*, 1993, 362, 59-62; Sreedharan et al., *Science*, 2008, 319, 1668-1672; Vance et al., *Brain*, 2009, 129, 868-876). Recently, linkage analysis of kindreds involving multiple cases of ALS, FTD, and ALS-FTD had suggested that there was an important locus for the disease on the short arm of chromosome 9 (Boxer et al., *J. Neurol. Neurosurg. Psychiatry*, 2011, 82, 196-203; Morita et al., *Neurology*, 2006, 66, 839-844; Pearson et al. *J. Nerol.*, 2011, 258, 647-655; Vance et al., *Brain*, 2006, 129, 868-876). This mutation has been found to be the most common genetic cause of ALS and FTD. It is postulated that the ALS-FTD causing mutation is a large hexanucleotide (GGGGCC) repeat expansion in the first intron of the C9ORF72 gene (Renton et al., *Neuron*, 2011, 72, 257-268; DeJesus-Hernandez et al., *Neuron*, 2011, 72, 245-256). A founder haplotype, covering the C9ORF72 gene, is present in the majority of cases linked to this region (Renton et al., *Neuron*, 2011, 72, 257-268). This locus on chromosome 9p21 accounts for nearly half of familial ALS and nearly one-quarter of all ALS cases in a cohort of 405 Finnish patients (Laaksovirta et al, *Lancet Neurol.*, 2010, 9, 978-985).

There are currently no effective therapies to treat such neurodegenerative diseases. Therefore, it is an object to provide compositions and methods for the treatment of such neurodegenerative diseases.

Summary

Provided herein are compositions and methods for modulating expression and levels of C9ORF72 antisense transcript in cells, tissues, and animals. In certain embodiments, C9ORF72 antisense transcript specific inhibitors modulate expression of C9ORF72 antisense transcript. In certain embodiments, C9ORF72 antisense transcript specific inhibitors are nucleic acids, proteins, or small molecules. In certain embodiments, C9ORF72 antisense transcript specific inhibitors are antisense compounds. In certain embodiments, C9ORF72 antisense compounds are modified oligonucleotides. In certain embodiments, the modified oligonucleotides are single-stranded.

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, C9ORF72 antisense transcript levels are reduced. In certain embodiments, C9ORF72 antisense transcript associated RAN translation products are reduced. In certain embodiments, the C9ORF72 antisense transcript associated RAN translation products are poly-(proline-alanine), poly-(proline-arginine), and poly-(proline-glycine). In certain embodiments, the C9ORF72 antisense

transcript contains a hexanucleotide repeat expansion. In certain embodiments, the hexanucleotide repeat is transcribed in the antisense direction from the C9ORF72 gene. In certain embodiments, the hexanucleotide repeat expansion is associated with a C9ORF72 associated disease. In certain embodiments, the hexanucleotide repeat expansion is associated with a C9ORF72 hexanucleotide repeat expansion associated disease. In certain embodiments, the hexanucleotide repeat expansion comprises at least 30 GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC repeats. In certain embodiments, the hexanucleotide repeat expansion comprises more than 30 GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC repeats. In certain embodiments, the hexanucleotide repeat expansion comprises more than 100 GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC repeats. In certain embodiments, the hexanucleotide repeat expansion comprises more than 500 GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC repeats. In certain embodiments, the hexanucleotide repeat expansion comprises at least 1000 GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC repeats. In certain embodiments, the hexanucleotide repeat expansion is associated with nuclear foci. In certain embodiments, C9ORF72 antisense transcript associated RAN translation products are associated with nuclear foci. In certain embodiments, the antisense transcript associated RAN translation products are poly-(proline-alanine) and/or poly-(proline-arginine). In certain embodiments, the compositions and methods described herein are useful for reducing C9ORF72 antisense transcript levels, C9ORF72 antisense transcript associated RAN translation products, and nuclear foci. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods useful for preventing, treating, ameliorating, and slowing progression of diseases and conditions associated with C9ORF72. In certain embodiments, such diseases and conditions associated with C9ORF72 are neurodegenerative diseases. In certain embodiments, the neurodegenerative disease is amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, or olivopontocerebellar degeneration (OPCD).

Such diseases and conditions can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of a neurodegenerative disease, and, in particular, ALS and FTD, include genetic predisposition and older age.

In certain embodiments, methods of treatment include administering a C9ORF72 antisense transcript specific inhibitor to an individual in need thereof. In certain embodiments, the C9ORF72 antisense transcript specific inhibitor is a nucleic acid. In certain embodiments, the nucleic acid is an antisense compound. In certain embodiments, the antisense compound is a modified

oligonucleotide. In certain embodiments, the antisense oligonucleotide is complementary to a C9ORF72 antisense transcript.

Detailed Description

5 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
10 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
15 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
20 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
25 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-methoxy-ethyl modification of the 2’ position of a furanose ring. A 2’-O-methoxyethyl modified
30 sugar is a modified sugar.

“2’-MOE nucleoside” (also 2’-O-methoxyethyl nucleoside) means a nucleoside comprising a 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’
5 position. A 5-methylcytosine is a modified nucleobase.

“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
10 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
15 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
20 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
25 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 product encoded by such target nucleic acid.

“Antisense compound” means an oligomeric compound that is capable of undergoing
30 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.

5 “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.

10 “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.

15 “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.

20 “C9ORF72 antisense transcript” means transcripts produced from the non-coding strand (also antisense strand and template strand) of the C9ORF72 gene. The C9ORF72 antisense transcript differs from the canonically transcribed “C9ORF72 sense transcript”, which is produced from the coding strand (also sense strand) of the C9ORF72 gene.

25 “C9ORF72 antisense transcript associated RAN translation products” means aberrant peptide or di-peptide polymers translated through RAN translation (i.e., repeat-associated, and non-ATG-dependent translation). In certain embodiments, the C9ORF72 antisense transcript associated RAN translation products are any of poly-(proline-alanine), poly-(proline-arginine), and poly-(proline-glycine).

30 “C9ORF72 antisense transcript specific inhibitor” refers to any agent capable of specifically inhibiting the expression of C9ORF72 antisense transcript and/or its expression products at the molecular level. For example, C9ORF72 specific antisense transcript inhibitors include nucleic acids (including antisense compounds), siRNAs, aptamers, antibodies, peptides, small molecules,

and other agents capable of inhibiting the expression of C9ORF72 antisense transcript and/or its expression products, such as C9ORF72 antisense transcript associated RAN translation products.

“C9ORF72 associated disease” means any disease associated with any C9ORF72 nucleic acid or expression product thereof, regardless of which DNA strand the C9ORF72 nucleic acid or expression product thereof is derived from. Such diseases may include a neurodegenerative disease. Such neurodegenerative diseases may include ALS and FTD.

“C9ORF72 foci” means nuclear foci comprising a C9ORF72 transcript. In certain embodiments, a C9ORF72 foci comprises at least one C9ORF72 sense transcript (herein “C9ORF72 sense foci”). In certain embodiments, C9ORF72 sense foci comprise C9ORF72 sense transcripts comprising any of the following hexanucleotide repeats: GGGGCC, GGGGGG, GGGGGC, and/or GGGGCG. In certain embodiments, a C9ORF72 foci comprises at least one C9ORF72 antisense transcript (herein “C9ORF72 antisense foci”). In certain embodiments, C9ORF72 antisense foci comprise C9ORF72 antisense transcripts comprising any of the following hexanucleotide repeats: GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC. In certain embodiments, C9ORF72 foci comprise both C9ORF72 sense transcripts and C9ORF72 antisense transcripts.

“C9ORF72 hexanucleotide repeat expansion associated disease” means any disease associated with a C9ORF72 nucleic acid containing a hexanucleotide repeat expansion. In certain embodiments, the hexanucleotide repeat expansion may comprise any of the following hexanucleotide repeats: GGGGCC, GGGGGG, GGGGGC, GGGGCG, GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC. In certain embodiments, the hexanucleotide repeat is repeated at least 30 times, more than 30 times, more than 100 times, more than 500 times, or more than 1000 times. Such diseases may include a neurodegenerative disease. Such neurodegenerative diseases may include ALS and FTD.

“C9ORF72 nucleic acid” means any nucleic acid derived from the C9ORF72 locus, regardless of which DNA strand the C9ORF72 nucleic acid is derived from. In certain embodiments, a C9ORF72 nucleic acid includes a DNA sequence encoding C9ORF72, an RNA sequence transcribed from DNA encoding C9ORF72 including genomic DNA comprising introns and exons (i.e., pre-mRNA), and an mRNA sequence encoding C9ORF72. “C9ORF72 mRNA” means an mRNA encoding a C9ORF72 protein. In certain embodiments, a C9ORF72 nucleic acid includes transcripts produced from the coding strand of the C9ORF72 gene. C9ORF72 sense transcripts are examples of C9ORF72 nucleic acids. In certain embodiments, a C9ORF72 nucleic

acid includes transcripts produced from the non-coding strand of the C9ORF72 gene. C9ORF72 antisense transcripts are examples of C9ORF72 nucleic acids.

“C9ORF72 pathogenic associated mRNA variant” means the C9ORF72 mRNA variant processed from a C9ORF72 pre-mRNA variant containing the hexanucleotide repeat. A C9ORF72 pre-mRNA contains the hexanucleotide repeat when transcription of the pre-mRNA begins in the region from the start site of exon 1A to the start site of exon 1B, e.g., nucleotides 1107 to 1520 of the genomic sequence (SEQ ID NO: 2, the complement of GENBANK Accession No. NT_008413.18 truncated from nucleosides 27535000 to 27565000). In certain embodiments, the level of a C9ORF72 pathogenic associated mRNA variant is measured to determine the level of a C9ORF72 pre-mRNA containing the hexanucleotide repeat in a sample.

“C9ORF72 transcript” means a RNA transcribed from C9ORF72. In certain embodiments, a C9ORF72 transcript is a C9ORF72 sense transcript. In certain embodiments, a C9ORF72 transcript is a C9ORF72 antisense transcript.

“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
5 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
10 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
15 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
20 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
25 condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual’s medical condition, and other relevant factors.

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

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

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

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

“Hexanucleotide repeat expansion” means a series of six bases (for example, GGGGCC, GGGGGG, GGGGGC, GGGGCG, GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC) repeated at least twice. In certain embodiments, the hexanucleotide repeat expansion may be located in intron 1 of a C9ORF72 nucleic acid. In certain embodiments, the hexanucleotide repeat may be transcribed in the antisense direction from the C9ORF72 gene. In certain embodiments, a pathogenic hexanucleotide repeat expansion includes more than 30, more than 100, more than 500, or more than 1000 repeats of GGGGCC, GGGGGG, GGGGGC, GGGGCG, GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC in a C9ORF72 nucleic acid and is associated with disease. In certain embodiments, the repeats are consecutive. In certain embodiments, the repeats are interrupted by 1 or more nucleobases. In certain embodiments, a wild-type hexanucleotide repeat expansion includes 30 or fewer repeats of GGGGCC, GGGGGG, GGGGGC, GGGGCG, GGCCCC, CCCCCC, GCCCCC, and/or CGCCCC in a C9ORF72 nucleic acid. In certain embodiments, the repeats are consecutive. In certain embodiments, the repeats are interrupted by 1 or more nucleobases.

“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.

“Identifying an animal having a C9ORF72 associated disease” means identifying an animal having been diagnosed with a C9ORF72 associated disease or predisposed to develop a C9ORF72 associated disease. Individuals predisposed to develop a C9ORF72 associated disease include those having one or more risk factors for developing a C9ORF72 associated disease, including, having a personal or family history or genetic predisposition of one or more C9ORF72 associated diseases. In certain embodiments, the C9ORF72 associated disease is a C9ORF72 hexanucleotide repeat expansion associated disease. 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.

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

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

"Inhibiting expression of a C9ORF72 antisense transcript" means reducing the level or expression of a C9ORF72 antisense transcript and/or its expression products (e.g., RAN translation products). In certain embodiments, C9ORF72 antisense transcripts are inhibited in the presence of an antisense compound targeting a C9ORF72 antisense transcript, including an antisense
10 oligonucleotide targeting a C9ORF72 antisense transcript, as compared to expression of C9ORF72 antisense transcript levels in the absence of a C9ORF72 antisense compound, such as an antisense oligonucleotide.

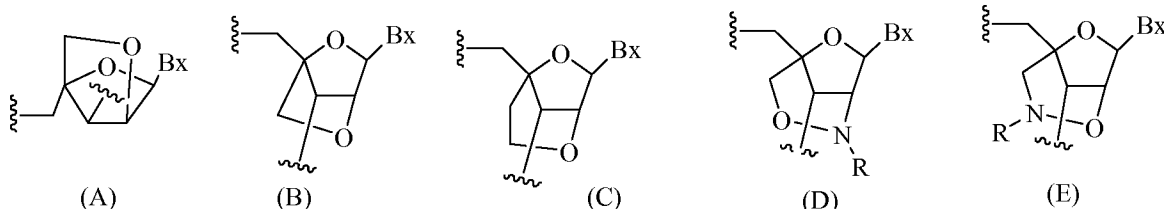
"Inhibiting expression of a C9ORF72 sense transcript" means reducing the level or expression of a C9ORF72 sense transcript and/or its expression products (e.g., a C9ORF72 mRNA and/or protein). In certain embodiments, C9ORF72 sense transcripts are inhibited in the presence of an antisense compound targeting a C9ORF72 sense transcript, including an antisense
15 oligonucleotide targeting a C9ORF72 sense transcript, as compared to expression of C9ORF72 sense transcript levels in the absence of a C9ORF72 antisense compound, such as an antisense oligonucleotide.

20 "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.

25 "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
30 (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, 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, a heterocycle radical, a substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5 - C_7 alicyclic radical, substituted C_5 - C_7 alicyclic radical, halogen, OJ_1 , NJ_1J_2 , SJ_1 , N_3 , $COOJ_1$, acyl ($C(=O)-H$), substituted acyl, CN, sulfonyl ($S(=O)_2-J_1$), or sulfoxyl ($S(=O)-J_1$); and each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)-H$), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted C_1 - C_{12} aminoalkyl or a protecting group.

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'- 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_1)-2' and 4'- CH_2 -N(R_1)-O-2'- bridges, wherein each R_1 and R_2 is, independently, H, a protecting group or C_1 - C_{12} 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'- CH_2 -O-2') bridge to form the bicyclic sugar moiety. The bridge can also be a methylene ($-CH_2-$) group connecting the 2' oxygen atom and the 4' carbon atom, for which the term methyleneoxy (4'- CH_2 -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'- CH_2CH_2 -O-2') LNA is used. α -L- methyleneoxy (4'- CH_2 -O-2'), an isomer of methyleneoxy (4'- CH_2 -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.

5 “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).

10 “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.

15 “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 nucleoside in an antisense compound.

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

20 “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.

25 “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.

30 “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.

5 “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
10 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
15 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
20 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.

25 “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
30 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.

5 “Pharmaceutical agent” means a substance that provides a therapeutic benefit when administered to an individual. In certain embodiments, an antisense oligonucleotide targeted to C9ORF72sense transcript is a pharmaceutical agent. In certain embodiments, an antisense oligonucleotide targeted to C9ORF72antisense transcript is a pharmaceutical agent.

10 “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.

15 “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.

20 “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.

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

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

30 “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.

5 “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
10 nervous system abnormalities, and myopathies.

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

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

15 “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 is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays and
20 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.

25 “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.

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

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

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

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

“Unmodified nucleobases” means the purine bases adenine (A) and guanine (G), and the pyrimidine bases (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).

“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 modulating expression and levels of C9ORF72 antisense transcript. Certain embodiments provide methods, compounds, and compositions for inhibiting expression and levels of C9ORF72 antisense transcript.

Certain embodiments provide antisense compounds targeted to a C9ORF72 antisense transcript. In certain embodiments, the C9ORF72 antisense transcript is the sequence set forth in SEQ ID NO: 11, which is a sequence that is complementary to nucleotides 1159 to 1929 of SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_008413.18 truncated from nucleotides 27535000 to 27565000).

Certain embodiments provide methods, compounds, and compositions for the treatment, prevention, amelioration, or slowing progression of disease associated with C9ORF72 antisense transcript in an individual in need thereof. Also contemplated are methods for the preparation of a medicament for the treatment, prevention, amelioration, or slowing progression of a disease associated with C9ORF72 antisense transcript. C9ORF72 antisense transcript diseases include

amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, or olivopontocerebellar degeneration (OPCD).

The present disclosure provides the following non-limiting numbered embodiments:

Embodiment 1. A compound comprising a modified oligonucleotide consisting of 12-
5 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 consecutive nucleobases of any of the nucleobases sequences of SEQ ID NOs 42-
80.

Embodiment 2. The compound of embodiment 1, wherein the modified
10 oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least
91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%,
at least 99%, or 100% complementary to a C9ORF72 antisense transcript.

Embodiment 3. The compound of embodiment 2, wherein the C9ORF72 antisense
transcript has the nucleobase sequence of SEQ ID NO: 11.

Embodiment 4. The compound of any of embodiments 1-3, wherein the modified
15 oligonucleotide is a single-stranded modified oligonucleotide.

Embodiment 5. The compound of any of embodiments 1-4, wherein the modified
oligonucleotide comprises at least one modified internucleoside linkage.

Embodiment 6. The compound of any of embodiment 5, wherein the at least one
20 modified internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 7. The compound of embodiments 5 or 6, wherein the modified
oligonucleotide comprises at least one phosphodiester linkage.

Embodiment 8. The compound of embodiment 6, wherein each internucleoside
linkage is a phosphorothioate internucleoside linkage.

Embodiment 9. The compound of any of embodiments 1-8, wherein at least one
25 nucleoside of the modified oligonucleotide comprises a modified nucleobase.

Embodiment 10. The compound of embodiment 9, wherein the modified nucleobase is
a 5-methylcytosine.

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

Embodiment 12. The compound of embodiment 11, wherein each nucleoside of the

modified oligonucleotide comprises a modified sugar.

Embodiment 13. The compound of embodiments 11 or 12, wherein the modified sugar is a bicyclic sugar.

Embodiment 14. The compound of embodiment 13, wherein the bicyclic sugar
5 comprises a chemical bridge between the 4' and 2' positions of the sugar, wherein the chemical bridge is selected from: 4'-CH(R)-O-2' and 4'-(CH₂)₂-O-2', wherein R is independently selected from H, C₁-C₆ alkyl, and C₁-C₆ alkoxy.

Embodiment 15. The compound of embodiment 14, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is methyl.

10 Embodiment 16. The compound of embodiment 14, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is H.

Embodiment 17. The compound of embodiment 14, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is -CH₂-O-CH₃.

Embodiment 18. The compound of embodiments 11 or 12, wherein the modified sugar
15 comprises a 2'-O-methoxyethyl group.

Embodiment 19. The compound of any of embodiments 1-11 and 13-18, wherein the modified oligonucleotide is a gapmer.

Embodiment 20. The compound of embodiment 19, wherein the gapmer is a 5-10-5 MOE gapmer.

20 Embodiment 21. A composition comprising the compound of any preceding embodiment or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

Embodiment 22. The composition of embodiment 21 further comprising a C9ORF72 sense transcript specific inhibitor.

Embodiment 23. The composition of embodiment 22, wherein the C9ORF72 sense
25 transcript specific inhibitor is any of a nucleic acid, aptamer, antibody, peptide, or small molecule.

Embodiment 24. The composition of embodiment 23, wherein the nucleic acid is a single-stranded nucleic acid or a double-stranded nucleic acid.

Embodiment 25. The composition of embodiment 23, wherein the nucleic acid is a siRNA.

30 Embodiment 26. The composition of embodiment 22, wherein the C9ORF72 sense transcript inhibitor is an antisense compound.

Embodiment 27. The composition of embodiment 26, wherein the antisense compound

is an antisense oligonucleotide.

Embodiment 28. The composition of embodiment 26, wherein the antisense compound is a modified oligonucleotide.

Embodiment 29. The composition of embodiment 28, wherein the modified
5 oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to a C9ORF72 sense transcript.

Embodiment 30. The compound of embodiment 29, wherein the C9ORF72 sense transcript has the nucleobase sequence of SEQ ID NO: 1-10.

10 Embodiment 31. Use of the compound or composition of any preceding embodiment for the manufacture of a medicament for treating a neurodegenerative disease.

Embodiment 32. A method comprising administering to an animal the compound or composition of any preceding embodiment.

Embodiment 33. The method of embodiment 32, wherein the animal is a human.

15 Embodiment 34. The method of embodiments 32 or 33, wherein the compound prevents, treats, ameliorates, or slows progression of at least one symptom of a C9ORF72 associated disease.

Embodiment 35. The method of embodiment 34, wherein the at least one symptom is selected from among impaired motor function, difficulty with respiration, muscle weakness,
20 fasciculation and cramping of muscles, difficulty in projecting the voice, shortness of breath, difficulty in breathing and swallowing, inappropriate social behavior, lack of empathy, distractibility, changes in food preference, agitation, blunted emotions, neglect of personal hygiene, repetitive or compulsive behavior, and decreased energy and motivation.

Embodiment 36. The method of embodiment 34, wherein the C9ORF72 associated
25 disease is amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, or olivopontocerebellar degeneration (OPCD).

Embodiment 37. The method of embodiment 36, wherein the amyotrophic lateral sclerosis (ALS) is familial ALS.

30 Embodiment 38. The method of embodiment 36, wherein the amyotrophic lateral sclerosis (ALS) is sporadic ALS.

Embodiment 39. The method of any of embodiments 32-38, wherein the administering

reduces C9ORF72 antisense transcript associated RAN translation products.

Embodiment 40. The method of embodiment 39, wherein the C9ORF72 antisense transcript associated RAN translation products are any of poly-(proline-alanine), poly-(proline-arginine), and poly-(proline-glycine).

5 Embodiment 41. The method of any of embodiments 32-40, wherein the administering is parenteral administration.

Embodiment 42. The method of embodiment 41, wherein the parenteral administration is any of injection or infusion.

10 Embodiment 43. The method of embodiment 42, wherein the parenteral administration is directly into the central nervous system (CNS).

Embodiment 44. The method of embodiments 41 or 42, wherein the parenteral administration is any of intrathecal administration or intracerebroventricular administration.

Embodiment 45. The method of any of embodiments 32-44, wherein the administering reduces C9ORF72 antisense foci.

15 Embodiment 46. The method of any of embodiments 32-44, wherein the administering reduces C9ORF72 sense foci.

Antisense Compounds

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

25 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.

30 In certain embodiments, an antisense compound targeted to a C9ORF72 nucleic acid is 12 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits. In certain embodiments, the antisense compound is 8 to 80, 12 to 50, 15 to 30, 18 to 24, 19 to 22, or 20 linked subunits. In certain embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14,

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

5 oligonucleotide, and the linked subunits are nucleosides.

In certain embodiments antisense oligonucleotides targeted to a C9ORF72 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 a C9ORF72 nucleic acid may have two subunits deleted from the 5' end, or
10 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
15 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
20 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
25 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.

30 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*.

Maier 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
5 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.

10 *Antisense Compound Motifs*

In certain embodiments, antisense compounds targeted to a C9ORF72 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.

15 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.

20 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,
25 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
30 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'). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X"

represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap segment is positioned immediately adjacent to each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing
5 segment and gap segment, or the gap segment and the 3' wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleotides. Thus, gapmers described herein include, but are not limited to, for
10 example 5-10-5, 5-10-4, 4-10-4, 4-10-3, 3-10-3, 2-10-2, 5-9-5, 5-9-4, 4-9-5, 5-8-5, 5-8-4, 4-8-5, 5-7-5, 4-7-5, 5-7-4, or 4-7-4.

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

In certain embodiments, an antisense compound targeted to a C9ORF72 nucleic acid has a gap-narrowed motif. In certain embodiments, a gap-narrowed antisense oligonucleotide targeted to a C9ORF72 nucleic acid has a gap segment of 9, 8, 7, or 6 2'-deoxynucleotides positioned immediately adjacent to and between wing segments of 5, 4, 3, 2, or 1 chemically modified
20 nucleosides. In certain embodiments, the chemical modification comprises a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4' to 2' bridge selected from among: 4'-(CH₂)_n-O-2' bridge, wherein n is 1 or 2; and 4'-CH₂-O-CH₂-2'. In certain embodiments, the bicyclic sugar is comprises a 4'-CH(CH₃)-O-2' bridge. In certain embodiments, the chemical modification comprises a non-bicyclic 2'-modified sugar moiety. In certain embodiments, the non-bicyclic 2'-
25 modified sugar moiety comprises a 2'-O-methylethyl group or a 2'-O-methyl group.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode C9ORF72 include, without limitation, the following: the complement of GENBANK Accession No. NM_001256054.1 (incorporated herein as SEQ ID NO:
30 1), GENBANK Accession No. NT_008413.18 truncated from nucleobase 27535000 to 27565000 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. BQ068108.1 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. NM_018325.3 (incorporated herein as SEQ

ID NO: 4), GENBANK Accession No. DN993522.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. NM_145005.5 (incorporated herein as SEQ ID NO: 6), GENBANK Accession No. DB079375.1 (incorporated herein as SEQ ID NO: 7), GENBANK Accession No. BU194591.1 (incorporated herein as SEQ ID NO: 8), Sequence Identifier 4141_014_A
5 (incorporated herein as SEQ ID NO: 9), and Sequence Identifier 4008_73_A (incorporated herein as SEQ ID NO: 10).

Nucleotide sequences that encode the C9ORF72 antisense transcript include, without limitation, the following: SEQ ID NO: 11 is a sequence that is complementary to nucleotides 1159 to 1929 of SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_008413.18 truncated
10 from nucleotides 27535000 to 27565000).

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
15 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,
20 or other defined nucleic acid region. The structurally defined regions for C9ORF72 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.

25 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.

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

nucleotides. In certain embodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceding values. In certain embodiments, target segments within a target region
5 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
10 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
15 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 a target region. In certain embodiments, reductions in C9ORF72 mRNA levels are indicative of inhibition of C9ORF72 expression. Reductions in levels
20 of a C9ORF72 protein are also indicative of inhibition of target mRNA expression. Reduction in the presence of expanded C9ORF72 RNA foci are indicative of inhibition of C9ORF72 expression. Further, phenotypic changes are indicative of inhibition of C9ORF72 expression. For example, improved motor function and respiration may be indicative of inhibition of C9ORF72 expression.

25 *Hybridization*

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a C9ORF72 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.

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

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a C9ORF72 nucleic acid.

5 *Complementarity*

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a C9ORF72 nucleic acid).

10 Non-complementary nucleobases between an antisense compound and a C9ORF72 nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a C9ORF72 nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

15 In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a C9ORF72 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.

20 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
25 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
30 PowerBLAST programs known in the art (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence

Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (*i.e.*, 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a C9ORF72 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, “fully complementary” means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be “fully complementary” to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a C9ORF72 nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-

complementary nucleobase(s) relative to a target nucleic acid, such as a C9ORF72 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
5 (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
10 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
15 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.

20

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
25 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
30 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
5 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,
10 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
15 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
20 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
25 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
30 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, phosphodiester, phosphotriester, methylphosphonate, phosphoramidate, and phosphorothioate. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a C9ORF72 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. In certain embodiments, the antisense compounds targeted to a C9ORF72 nucleic acid comprise at least one phosphodiester linkage and at least one phosphorothioate linkage.

Modified Sugar Moieties

Antisense compounds of the invention can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise 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-CH₂-C(=O)-N(R₁)-(CH₂)₂-N(R_m)(R_n), where each R₁, R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleic acids (BNAs) 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 BNA nucleosides wherein the bridge comprises one of the formulas: 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' and 4'-CH(CH₂OCH₃)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2' (and analogs thereof see PCT/US2008/068922 published as WO/2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2' (and analogs thereof see PCT/US2008/064591 published as WO/2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (see Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof see PCT/US2008/066154 published as WO 2008/154401, published on December 8, 2008).

Further bicyclic nucleosides have been reported in published literature (see for example: Srivastava *et al.*, *J. Am. Chem. Soc.*, 2007, 129(26) 8362-8379; Frieden *et al.*, *Nucleic Acids Research*, 2003, 21, 6365-6372; Elayadi *et al.*, *Curr. Opinion Invens. Drugs*, 2001, 2, 558-561; Braasch *et al.*, *Chem. Biol.*, 2001, 8, 1-7; Orum *et al.*, *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; Wahlestedt *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Singh *et al.*, *Chem. Commun.*, 1998, 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630; Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039;

U.S. Patents Nos.: 7,399,845; 7,053,207; 7,034,133; 6,794,499; 6,770,748; 6,670,461; 6,525,191; 6,268,490; U.S. Patent Publication Nos.: US2008-0039618; US2007-0287831; US2004-0171570; U.S. Patent Applications, Serial Nos.: 12/129,154; 61/099,844; 61/097,787; 61/086,231; 61/056,564; 61/026,998; 61/026,995; 60/989,574; International applications WO 2007/134181; WO
 5 2005/021570; WO 2004/106356; and PCT International Applications Nos.: PCT/US2008/068922; PCT/US2008/066154; and PCT/US2008/064591). 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).

10 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, “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
 15 the furanose ring connects the 2’ carbon atom and the 4’ carbon atom of the sugar ring.

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’ carbon atoms of the pentofuranosyl sugar moiety including without limitation, bridges comprising 1 or from 1 to 4
 20 linked groups independently selected from $-\text{C}(\text{R}_a)(\text{R}_b)]_n-$, $-\text{C}(\text{R}_a)=\text{C}(\text{R}_b)-$, $-\text{C}(\text{R}_a)=\text{N}-$, $-\text{C}(=\text{NR}_a)-$, $-\text{C}(=\text{O})-$, $-\text{C}(=\text{S})-$, $-\text{O}-$, $-\text{Si}(\text{R}_a)_2-$, $-\text{S}(=\text{O})_x-$, and $-\text{N}(\text{R}_a)-$; 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 ,
 25 COOJ_1 , acyl ($\text{C}(=\text{O})-\text{H}$), substituted acyl, CN, sulfonyl ($\text{S}(=\text{O})_2-\text{J}_1$), or sulfoxyl ($\text{S}(=\text{O})-\text{J}_1$); 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 ($\text{C}(=\text{O})-\text{H}$), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted C_1 - C_{12} aminoalkyl or a protecting group.

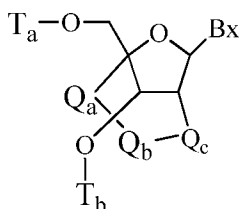
30 In certain embodiments, the bridge of a bicyclic sugar moiety is $-\text{C}(\text{R}_a)(\text{R}_b)]_n-$, $-\text{C}(\text{R}_a)(\text{R}_b)]_n-\text{O}-$, $-\text{C}(\text{R}_a\text{R}_b)-\text{N}(\text{R})-\text{O}-$ or $-\text{C}(\text{R}_a\text{R}_b)-\text{O}-\text{N}(\text{R})-$. In certain embodiments, the bridge is

4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R)-2' and 4'-CH₂-N(R)-O-2'- wherein each R is, independently, H, a protecting group or C₁-C₁₂ alkyl.

In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-(CH₂)-O-2' bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH₂-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 those having a 4' to 2' bridge wherein such bridges include without limitation, α-L-4'-(CH₂)-O-2', β-D-4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R)-2', 4'-CH₂-N(R)-O-2', 4'-CH(CH₃)-O-2', 4'-CH₂-S-2', 4'-CH₂-N(R)-2', 4'-CH₂-CH(CH₃)-2', and 4'-(CH₂)₃-2', wherein R is H, a protecting group or C₁-C₁₂ alkyl.

In certain embodiment, bicyclic nucleosides have the formula:



wherein:

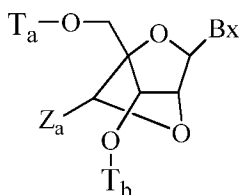
15 Bx is a heterocyclic base moiety;

-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

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

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

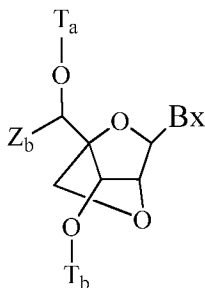
Bx is a heterocyclic base moiety;

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

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

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

10 In certain embodiments, bicyclic nucleosides have the formula:



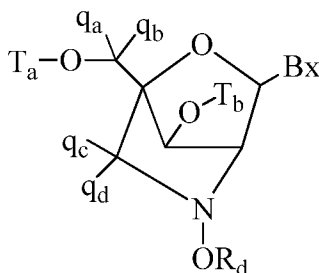
wherein:

Bx is a heterocyclic base moiety;

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

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

In certain embodiments, bicyclic nucleosides have the formula:



20 wherein:

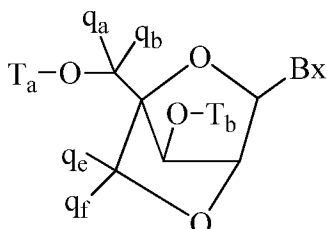
Bx is a heterocyclic base moiety;

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

R_d is C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

each q_a, q_b, q_c and q_d is, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, acyl, substituted acyl, C₁-C₆ aminoalkyl or substituted C₁-C₆ aminoalkyl;

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

Bx is a heterocyclic base moiety;

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

q_a, q_b, q_c and q_f 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, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k or N(H)C(=S)NJ_jJ_k;

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

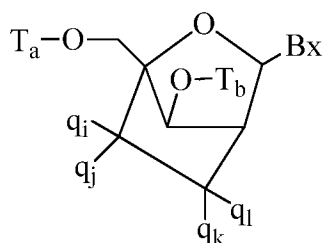
q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl or substituted C₁-C₁₂ alkyl.

The synthesis and preparation of adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil bicyclic nucleosides having a 4'-CH₂-O-2' bridge, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., *Tetrahedron*, 1998, 54, 3607-3630). The synthesis of bicyclic nucleosides has also been described in WO 98/39352 and WO 99/14226.

Analogous of various bicyclic nucleosides that have 4' to 2' bridging groups such as 4'-CH₂-O-2' and 4'-CH₂-S-2', have also been prepared (Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222). Preparation of oligodeoxyribonucleotide duplexes comprising bicyclic nucleosides for use as substrates for nucleic acid polymerases has also been described (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'-methylamino-BNA's have been prepared and the thermal

stability of their duplexes with complementary RNA and DNA strands has been previously reported.

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

5 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;

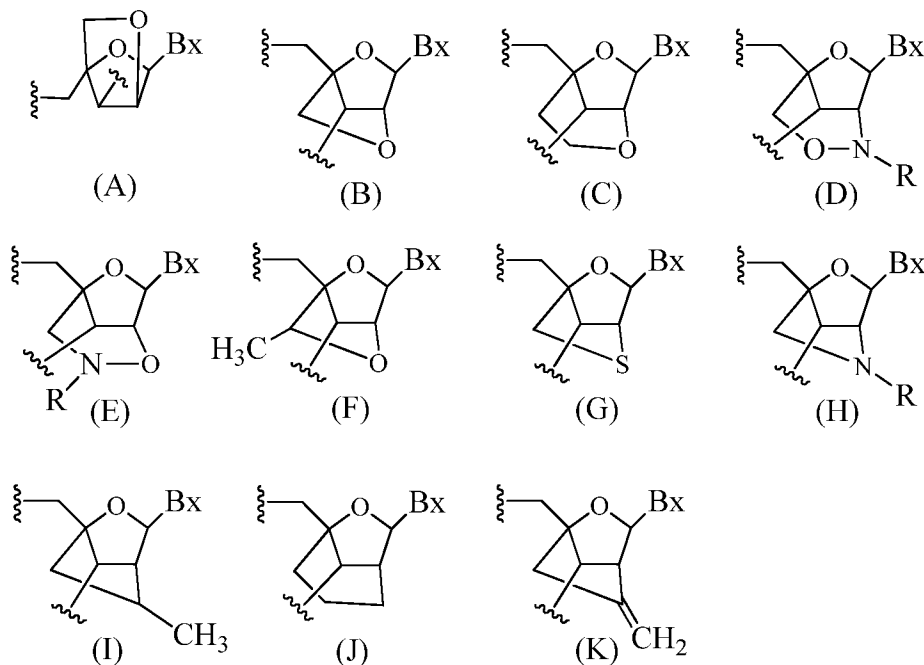
each qi, qj, qk and ql is, independently, H, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k or N(H)C(=S)NJ_jJ_k; and

qi and qj or qi and qk together are =C(q_g)(q_h), wherein q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl or substituted C₁-C₁₂ alkyl.

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

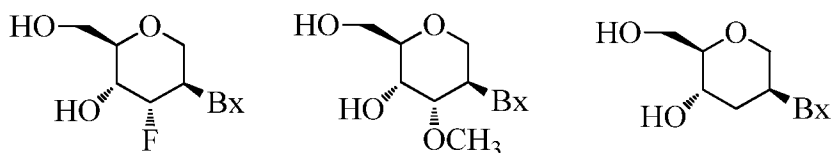
In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-methyleneoxy (4'-CH₂-O-2') BNA, (B) β-D-methyleneoxy (4'-CH₂-O-2') BNA, (C) ethyleneoxy (4'-(CH₂)₂-O-2') BNA, (D) aminooxy (4'-CH₂-O-N(R)-2') BNA, (E) oxyamino (4'-CH₂-N(R)-O-2') BNA, (F) methyl(methyleneoxy) (4'-CH(CH₃)-O-2') BNA (also referred to as constrained ethyl or cEt), (G) methylene-thio (4'-CH₂-S-2') BNA, (H) methylene-amino (4'-CH₂-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH₂-CH(CH₃)-2') BNA, (J) propylene carbocyclic (4'-(CH₂)₃-2') BNA, and

25 (K) vinyl BNA as depicted below.

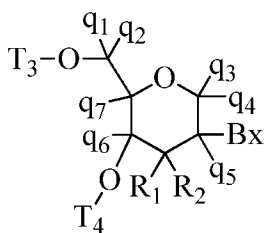


wherein Bx is the base moiety and R is, independently, H, a protecting group, C₁-C₆ alkyl or C₁-C₆ alkoxy.

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



In certain embodiment, sugar surrogates are selected having the formula:



wherein:

Bx is a heterocyclic base moiety;

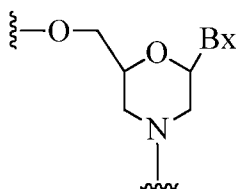
T₃ and T₄ are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the oligomeric compound or one of T₃ and T₄ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to an oligomeric compound or oligonucleotide and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked
 5 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

one of R₁ and R₂ is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ₃C(=X)NJ₁J₂ and CN, wherein X
 10 is O, S or NJ₁ and each J₁, J₂ and J₃ is, independently, H or C₁-C₆ alkyl.

In certain embodiments, 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 are provided wherein one of R₁ and R₂ is F. In certain embodiments, R₁ is fluoro and R₂ is H; R₁ is methoxy and R₂ is H, and
 15 R₁ is methoxyethoxy and R₂ is H.

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

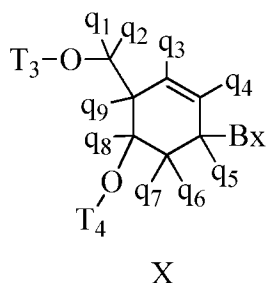


In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as "modified morpholinos."

20 Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic

acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH₂-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g., Srivastava et al., J. Am. Chem. Soc.*
 5 2007, 129(26), 8362-8379).

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



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

Bx is a heterocyclic base moiety;

T₃ and T₄ are each, independently, an internucleoside linking group linking the cyclohexenyl nucleoside analog to an antisense compound or one of T₃ and T₄ is an internucleoside linking group

linking the tetrahydropyran nucleoside analog to an antisense compound and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked conjugate group, or a 5'-or 3'-terminal group; and

q₁, q₂, q₃, q₄, q₅, q₆, q₇, q₈ and q₉ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₂-C₆ alkynyl or other
5 sugar substituent group.

Many other monocyclic, bicyclic and tricyclic ring systems are known in the art and are suitable as sugar surrogates that can be used to modify nucleosides for incorporation into oligomeric compounds as provided herein (see for example review article: Leumann, Christian J. *Bioorg. & Med. Chem.*, 2002, 10, 841-854). Such ring systems can undergo various additional substitutions to
10 further enhance their activity.

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
15 unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: O[(CH₂)_nO]_mCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nF, O(CH₂)_nONH₂, OCH₂C(=O)N(H)CH₃, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C₁-C₁₂ alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or
20 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'-
25 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-
30 504; 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, "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-CH₂-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl. 2'-modified nucleosides may further comprise other modifications, for example at other positions of the sugar and/or at the nucleobase.

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

As used herein, "2'-OMe" or "2'-OCH₃", "2'-O-methyl" or "2'-methoxy" 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.

Methods for the preparations of modified sugars are well known to those skilled in the art. Some representative U.S. patents that teach the preparation of such modified sugars include without limitation, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 20 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,670,633; 5,700,920; 5,792,847 and 6,600,032 and International Application PCT/US2005/019219, filed June 2, 2005 and published as WO 2005/121371 on December 22, 2005, and each of which is herein incorporated by reference in its entirety.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked 25 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).

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.

30 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.

5 *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
10 administered.

An antisense compound targeted to a C9ORF72 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
15 parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a C9ORF72 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
20 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
25 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.

30 *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 C9ORF72 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.

In general, cells are 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 are mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

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

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 a C9ORF72 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-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

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

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

25 Probes and primers are designed to hybridize to a C9ORF72 nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Strand Specific Semi-Quantitative PCR Analysis of Target RNA Levels

30 Analysis of specific, low abundance target RNA strand levels may be accomplished by reverse transcription, PCR, and gel densitometry analysis using the Gel Logic 200 Imaging System and Kodak MI software (Kodak Scientific Imaging Systems, Rochester, NY, USA) according to manufacturer's instructions.

RT-PCR reactions are carried out as taught in Ladd, P.D., et al, (Human Molecular Genetics, 2007, 16, 3174-3187) and in Sopher, B.L., et al, (Neuron, 2011, 70, 1071-1084) and such

methods are well known in the art.

The PCR amplification products are loaded onto gels, stained with ethidium bromide, and subjected to densitometry analysis. Mean intensities from regions of interest (ROI) that correspond to the bands of interest in the gel are measured.

5 Gene (or RNA) target quantities obtained by PCR are normalized using the expression level of a housekeeping gene whose expression is constant, such as GAPDH. Expression of the housekeeping gene (or RNA) is analyzed and measured using the same methods as the target.

Probes and primers are designed to hybridize to a C9ORF72 nucleic acid. Methods for designing RT-PCR probes and primers are well known in the art, and may include the use of
10 software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of C9ORF72 nucleic acids can be assessed by measuring C9ORF72 protein levels. Protein levels of C9ORF72 can be evaluated or quantitated in a variety of ways well
15 known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or
20 can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of mouse, rat, monkey, and human C9ORF72 are commercially available.

In vivo testing of antisense compounds

25 Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of C9ORF72 and produce phenotypic changes, such as, improved motor function and respiration. In certain embodiments, motor function is measured by rotarod, grip strength, pole climb, open field performance, balance beam, hindpaw footprint testing in the animal. In certain embodiments, respiration is measured by whole body plethysmograph,
30 invasive resistance, and compliance measurements 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
5 oligonucleotides, RNA is isolated from CNS tissue or CSF and changes in C9ORF72 nucleic acid expression are measured.

Targeting C9ORF72

Antisense oligonucleotides described herein may hybridize to a C9ORF72 nucleic acid
10 derived from either DNA strand. For example, antisense oligonucleotides described herein may hybridize to a C9ORF72 antisense transcript or a C9ORF72 sense transcript. Antisense oligonucleotides described herein may hybridize to a C9ORF72 nucleic acid in any stage of RNA processing. Described herein are antisense oligonucleotides that are complementary to a pre-mRNA or a mature mRNA. Additionally, antisense oligonucleotides described herein may hybridize to any
15 element of a C9ORF72 nucleic acid. For example, described herein are antisense oligonucleotides that are complementary to an exon, an intron, the 5' UTR, the 3' UTR, a repeat region, a hexanucleotide repeat expansion, a splice junction, an exon:exon splice junction, an exonic splicing silencer (ESS), an exonic splicing enhancer (ESE), exon 1a, exon 1b, exon 1c, exon 1d, exon 1e, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, exon 10, exon 11, intron 1, intron 2,
20 intron 3, intron 4, intron 5, intron 6, intron 7, intron 8, intron 9, or intron 10 of a C9ORF72 nucleic acid.

In certain embodiments, antisense oligonucleotides described herein hybridize to all variants of C9ORF72 derived from the sense strand. In certain embodiments, the antisense oligonucleotides described herein selectively hybridize to certain variants of C9ORF72 derived from
25 the sense strand. In certain embodiments, the antisense oligonucleotides described herein selectively hybridize to variants of C9ORF72 derived from the sense strand containing a hexanucleotide repeat expansion. In certain embodiments, the antisense oligonucleotides described herein selectively hybridize to pre-mRNA variants containing a hexanucleotide repeat. In certain embodiments, pre-mRNA variants of C9ORF72 containing a hexanucleotide repeat expansion
30 include SEQ ID NO: 1-3 and 6-10. In certain embodiments, such hexanucleotide repeat expansion comprises at least 24 repeats of any of GGGGCC, GGGGGG, GGGGGC, or GGGGCG.

In certain embodiments, the antisense oligonucleotides described herein inhibit expression of all variants of C9ORF72 derived from the sense strand. In certain embodiments, the antisense oligonucleotides described herein inhibit expression of all variants of C9ORF72 derived from the sense strand equally. In certain embodiments, the antisense oligonucleotides described herein preferentially inhibit expression of one or more variants of C9ORF72 derived from the sense strand. In certain embodiments, the antisense oligonucleotides described herein preferentially inhibit expression of variants of C9ORF72 derived from the sense strand containing a hexanucleotide repeat expansion. In certain embodiments, the antisense oligonucleotides described herein selectively inhibit expression of pre-mRNA variants containing the hexanucleotide repeat. In certain embodiments, the antisense oligonucleotides described herein selectively inhibit expression of C9ORF72 pathogenic associated mRNA variants. In certain embodiments, pre-mRNA variants of C9ORF72 containing a hexanucleotide repeat expansion include SEQ ID NO: 1-3 and 6-10. In certain embodiments, such hexanucleotide repeat expansion comprises at least 24 repeats of any of GGGGCC, GGGGGG, GGGGGC, or GGGGCG. In certain embodiments, the hexanucleotide repeat expansion forms C9ORF72 sense foci. In certain embodiments, antisense oligonucleotides described herein are useful for reducing C9ORF72 sense foci. C9ORF72 sense foci may be reduced in terms of percent of cells with foci as well as number of foci per cell.

C9ORF72 Features

Antisense oligonucleotides described herein may hybridize to any C9ORF72 nucleic acid at any state of processing within any element of the C9ORF72 gene. In certain embodiments, antisense oligonucleotides described herein may target the antisense transcript, e.g., SEQ ID NO: 11. In certain embodiments, antisense oligonucleotides described herein may hybridize to an exon, an intron, the 5' UTR, the 3' UTR, a repeat region, a hexanucleotide repeat expansion, a splice junction, an exon:exon splice junction, an exonic splicing silencer (ESS), an exonic splicing enhancer (ESE), exon 1a, exon 1b, exon 1c, exon 1d, exon 1e, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, exon 10, exon 11, intron 1, intron 2, intron 3, intron 4, intron 5, intron 6, intron 7, intron 8, intron 9, or intron 10. For example, antisense oligonucleotides may target any of the exons characterized below in Tables 1-5 described below. Antisense oligonucleotides described herein may also target nucleic acids not characterized below and such nucleic acid may be characterized in GENBANK. Moreover, antisense oligonucleotides described herein may also target elements other than exons and such elements as characterized in GENBANK.

Table 1

Functional Segments for NM_001256054.1 (SEQ ID NO: 1)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1C	1	158	1137	1294
exon 2	159	646	7839	8326
exon 3	647	706	9413	9472
exon 4	707	802	12527	12622
exon 5	803	867	13354	13418
exon 6	868	940	14704	14776
exon 7	941	1057	16396	16512
exon 8	1058	1293	18207	18442
exon 9	1294	1351	24296	24353
exon 10	1352	1461	26337	26446
exon 11	1462	3339	26581	28458

5

Table 2

Functional Segments for NM_018325.3 (SEQ ID NO: 4)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1B	1	63	1510	1572
exon 2	64	551	7839	8326
exon 3	552	611	9413	9472
exon 4	612	707	12527	12622
exon 5	708	772	13354	13418
exon 6	773	845	14704	14776
exon 7	846	962	16396	16512
exon 8	963	1198	18207	18442
exon 9	1199	1256	24296	24353
exon 10	1257	1366	26337	26446
exon 11	1367	3244	26581	28458

Table 3
Functional Segments for NM_145005.5 (SEQ ID NO: 6)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1A	1	80	1137	1216
exon 2	81	568	7839	8326
exon 3	569	628	9413	9472
exon 4	629	724	12527	12622
exon 5B (exon 5 into intron 5)	725	1871	13354	14500

5

Table 4
Functional Segments for DB079375.1 (SEQ ID NO: 7)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1E	1	35	1135	1169
exon 2	36	524	7839	8326
exon 3 (EST ends before end of full exon)	525	562	9413	9450

Table 5
Functional Segments for BU194591.1 (SEQ ID NO: 8)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1D	1	36	1241	1279
exon 2	37	524	7839	8326
exon 3	525	584	9413	9472
exon 4	585	680	12527	12622
exon 5B (exon 5 into intron 5)	681	798	13354	13465

10 *Certain Indications*

In certain embodiments, provided herein are methods 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, ALS or FTD. In certain embodiments, the individual has been identified as having a C9ORF72 associated disease. In certain embodiments, the individual has been identified as having a C9ORF72 hexanucleotide repeat expansion associated disease. In certain embodiments, provided herein are methods for prophylactically reducing C9ORF72 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 a C9ORF72 nucleic acid.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to a C9ORF72 nucleic acid is accompanied by monitoring of C9ORF72 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 a C9ORF72 nucleic acid results in reduction of C9ORF72 expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to a C9ORF72 nucleic acid results in improved motor function and respiration in an animal. In certain embodiments, administration of a C9ORF72 antisense compound improves motor function and respiration by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, administration of an antisense compound targeted to a C9ORF72 antisense transcript results in reduction of C9ORF72 antisense transcript expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to a C9ORF72 antisense transcript results in improved motor function and respiration in an animal. In certain embodiments, administration of a C9ORF72 antisense compound improves motor function and respiration by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of a C9ORF72 antisense compound reduces the number of cells with C9ORF72 antisense foci and/or the number of C9ORF72 antisense foci per cell.

In certain embodiments, administration of an antisense compound targeted to a C9ORF72 sense transcript results in reduction of a C9ORF72 sense transcript expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to a C9ORF72 sense transcript results in improved motor function and respiration in an animal. In certain
5
embodiments, administration of a C9ORF72 antisense compound improves motor function and respiration by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of a C9ORF72 antisense compound reduces the number of cells with C9ORF72 sense foci and/or the number of
10 C9ORF72 sense foci per cell.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to a C9ORF72 nucleic are used for the preparation of a medicament for treating a patient suffering or susceptible to a neurodegenerative disease including ALS and FTD.

15 *Certain Combination Therapies*

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

30 In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical

agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical agents are prepared separately.

5 In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition described herein include Riluzole (Rilutek), Lioresal (Lioresal), and Dextramipexole.

 In certain embodiments, pharmaceutical agents that may be co-administered with a C9ORF72 antisense transcript specific inhibitor described herein include, but are not limited to, an
10 additional C9ORF72 inhibitor. In certain embodiments, the co-administered pharmaceutical agent is administered prior to administration of a pharmaceutical composition described herein. In certain
embodiments, the co-administered pharmaceutical agent is administered following administration of a pharmaceutical composition described herein. In certain embodiments the co-administered
15 pharmaceutical agent is administered at the same time as a pharmaceutical composition described herein. In certain embodiments the dose of a co-administered pharmaceutical agent is the same as
the dose that would be administered if the co-administered pharmaceutical agent was administered alone. In certain embodiments the dose of a co-administered pharmaceutical agent is lower than the
dose that would be administered if the co-administered pharmaceutical agent was administered
20 alone. In certain embodiments the dose of a co-administered pharmaceutical agent is greater than
the dose that would be administered if the co-administered pharmaceutical agent was administered
alone.

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

30 *Certain Human Therapeutics*

 The human C9ORF72 antisense transcript specific antisense compounds described herein are human therapeutics. Various parameters of potency, efficacy, and/or tolerability are being

examined. Such parameters include *in vitro* inhibition of C9ORF72 antisense transcript; *in vitro* dose response (IC50); *in vivo* inhibition of C9ORF72 antisense transcript in a transgenic animal containing a human C9ORF72 transgene in relevant tissues (e.g., brain and/or spinal cord); and/or tolerability in mouse, rat, dog, and/or primate. Tolerability markers that may be measured include
5 blood and serum chemistry parameters, CSF chemistry parameters, body and organ weights, general observations and/or behavioral tests, and/or biochemical markers such as GFAP and/or AIF1. Acute or long term tolerability may be measured.

EXAMPLES

10 *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.

15

Example 1: Antisense inhibition of C9ORF72 antisense transcript with oligonucleotides

Antisense oligonucleotides (ASOs) targeting the human C9ORF72 antisense transcript were made and tested for inhibition of C9ORF72 antisense transcript *in vitro*. All of the ASOs in the tables below are 5-10-5 MOE gapmers, 20 nucleosides in length, wherein the central gap segment
20 consists of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction consisting of five nucleosides each. Each nucleoside in the 5' and 3' wing segments has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start" indicates the 5'-most nucleoside to which the gapmer is targeted in the target transcript sequence. "Stop"
25 indicates the 3'-most nucleoside to which the gapmer is targeted in the target transcript sequence. Each gapmer listed in the tables below is targeted to a putative antisense transcript sequence, designated herein as SEQ ID NO: 11. The sequence of SEQ ID NO: 11 is complementary to nucleotides 1159 to 1929 of SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_008413.18 truncated from nucleotides 27535000 to 27565000) except that SEQ ID NO: 11 has
30 two more six nucleotide repeats than SEQ ID NO: 2. The sequence of the hexanucleotide repeat is

GGCCCC in SEQ ID NO: 11 and GGGGCC in SEQ ID NO: 2. Thus, SEQ ID NO: 11 is 12 nucleotides longer than nucleotides 1159 to 1929 of SEQ ID NO: 2, to which it is complementary. Isis No. 129700 is a negative control ASO that does not target the C9ORF72 antisense transcript.

bEND cells were cultured in 24 well plates at 45,000-50,000 cells/well 24 hours before the first of two transfections. The cells were first transfected with 0.2 µg/well of a plasmid expressing the C9ORF72 antisense transcript (SEQ ID NO: 11) and 0.5 µL Lipofectamine 2000 (Life Technologies, Carlsbad, CA) in OptiMEM medium. Four to six hours later, the media was replaced. 24 hours after the first transfection (18-20 hours after media replacement), the bEND cells were transfected with 25 nM of an ASO listed in the tables below and 0.5 µL Lipofectamine 2000 in OptiMEM medium or with no ASO. Total RNA was isolated from the cells 24 hours after the second transfection using TRIzol (Life Technologies) according to the manufacturer's directions. Two DNase reactions were performed, one on the column during RNA purification, and one after purification using TURBO DNase (Life Technologies).

Strand specific RT-qPCR was performed on the isolated RNA to generate and amplify C9ORF72 antisense cDNA using one or two of three different primer sets, LTS01222, LTS01221, and C9ATS3'-1. The LTS01222 sequences are: RT primer: CGACTGGAGCACGAGGACACTGAAAAGATGACGCTTGGTGTGTCA (SEQ ID NO: 12), forward PCR primer: CCCACACCTGCTCTTGCTAGA (SEQ ID NO: 13), reverse PCR primer: CGACTGGAGCACGAGGACACTG (SEQ ID NO: 14), and probe: CCCAAAAGAGAAGCAACCGGGCA (SEQ ID NO: 15). The LTS01221 sequences are: RT primer: CGACTGGAGCACGAGGACACTGACGGCTGCCGGGAAGA (SEQ ID NO: 16), forward PCR primer: AGAAATGAGAGGGAAAGTAAAAATGC (SEQ ID NO: 17), reverse PCR primer: CGACTGGAGCACGAGGACACTG (SEQ ID NO: 18), and probe: AGGAGAGCCCCGCTTCTACCCG (SEQ ID NO: 19). The C9ATS3'-1 sequences are: RT primer: CGACTGGAGCACGAGGACACTGACGCTGAGGGTGAACAAGAA (SEQ ID NO: 20), forward PCR primer: GAGTTCCAGAGCTTGCTACAG (SEQ ID NO: 21), reverse PCR primer: CGACTGGAGCACGAGGACACTG (SEQ ID NO: 22), and probe: CTGCGGTTGTTTCCCTCCTTGTTT (SEQ ID NO: 23). RT-qPCR was also performed on the isolated RNA using Express One-Step Superscript qRT-PCR Kit (Life Technologies, Carlsbad, CA) according to manufacturer's instructions to generate and amplify GAPDH cDNA, as a control, using forward PCR primer: GGCAAATTCAACGGCACAGT (SEQ ID NO: 24), reverse PCR primer: GGGTCTCGCTCCTGGAAGAT (SEQ ID NO: 25), and probe:

AAGGCCGAGAATGGGAAGCTTGTCATC (SEQ ID NO: 26). The resulting C9ORF72 antisense transcript levels were normalized to GAPDH. These normalized values for C9ORF72 antisense transcript expression in cells treated with an ASO were then compared to the normalized values for C9ORF72 antisense transcript expression in control cells that were transfected with the C9ORF72 antisense plasmid but not an ASO. The results for each primer probe set are shown in the tables below as percent inhibition of C9ORF72 antisense transcript expression relative to the control cells that were not transfected with an ASO. A result of 0% inhibition indicates that the C9ORF72 antisense transcript levels were equal to that of control cells that were not transfected with an ASO. A negative value for % inhibition indicates that the C9ORF72 antisense transcript levels were higher than that of control cells that were not transfected with an ASO. A result of “n/a” indicates that the corresponding primer probe set was not used to analyze the indicated sample. The results show that many ASOs inhibited human C9ORF72 antisense transcript expression. The absolute inhibition results varied across different primer probe sets, but the relative potencies of the ASOs were very similar across different primer probe sets.

15

Table 1

Inhibition of C9ORF72 Antisense Transcript by Antisense Oligonucleotides

Isis No.	Start	Stop	Sequence	LTS 01222	LTS 01221	C9ATS 3'-1	SEQ ID No.
129700	n/a	n/a	TAGTGCGGACCTACCCACGA	-28	8	-21	41
661167	454	473	GGGGCCGGGGCCGGGGCCGG	31	n/a	n/a	42
	460	479					
	466	485					
661168	453	472	GGGCCGGGGCCGGGGCCGGG	41	n/a	n/a	43
	459	478					
	465	484					
661169	452	471	GGCCGGGGCCGGGGCCGGGG	51	n/a	n/a	44
	458	477					
	464	483					
661170	451	470	GCCGGGGCCGGGGCCGGGGC	54	n/a	n/a	45
	457	476					
	463	482					
661171	456	475	CCGGGGCCGGGGCCGGGGCC	59	n/a	n/a	46
	462	481					

661172	455	474	CGGGGCCGGGGCCGGGGCCG	24	n/a	n/a	47
	461	480					
664570	764	783	AGGAAAGAGAGGTGCGTCAA	n/a	52	67	48
664571	761	780	AAAGAGAGGTGCGTCAAACA	n/a	46	62	49
664572	758	777	GAGAGGTGCGTCAAACAGCG	n/a	43	67	50
664573	755	774	AGGTGCGTCAAACAGCGACA	n/a	35	60	51
664574	752	771	TGCGTCAAACAGCGACAAGT	n/a	38	63	52
664575	749	768	GTCAAACAGCGACAAGTTCC	n/a	44	54	53
664576	746	765	AAACAGCGACAAGTTCCGCC	n/a	19	52	54
664577	743	762	CAGCGACAAGTTCCGCCAC	n/a	-5	-27	55
664578	740	759	CGACAAGTTCCGCCACGTA	n/a	52	51	56
664579	737	756	CAAGTTCCGCCACGTAAAA	n/a	57	38	57
664580	734	753	GTTCCGCCACGTAAAAGAT	n/a	87	92	58
664581	731	750	CCGCCACGTAAAAGATGAC	n/a	62	72	59
664582	728	747	CCCACGTAAAAGATGACGCT	n/a	54	58	60
664583	725	744	ACGTAAAAGATGACGCTTGG	n/a	65	75	61
664584	722	741	TAAAAGATGACGCTTGGTGT	n/a	61	54	62
664585	719	738	AAGATGACGCTTGGTGTGTC	n/a	67	77	63
664586	716	735	ATGACGCTTGGTGTGTCAGC	n/a	71	72	64
664587	713	732	ACGCTTGGTGTGTCAGCCGT	n/a	45	49	65
664588	710	729	CTTGGTGTGTCAGCCGTCCC	n/a	34	57	66
664589	701	720	TCAGCCGTCCCTGCTGCCCG	n/a	35	40	67
664590	698	717	GCCGTCCCTGCTGCCCGGTT	n/a	17	22	68
664591	695	714	GTCCCTGCTGCCCGGTTGCT	n/a	60	70	69
664592	692	711	CCTGCTGCCCGGTTGCTTCT	n/a	60	83	70
664593	689	708	GCTGCCCGGTTGCTTCTCTT	n/a	41	74	71
664594	686	705	GCCCGGTTGCTTCTCTTTG	n/a	33	69	72
664596	656	675	CTAGCAAGAGCAGGTGTGGG	n/a	43	47	73
664599	647	666	GCAGGTGTGGGTTTAGGAGG	n/a	40	46	74
664600	644	663	GGTGTGGGTTTAGGAGGTGT	n/a	55	63	75
664611	587	606	GCTCTCACAGTACTCGCTGA	63	49	n/a	76
664613	581	600	ACAGTACTCGCTGAGGGTGA	79	48	n/a	77
664623	545	564	TAAAGATTAACCAGAAGAAA	64	52	n/a	78
664629	509	528	CCGCAGCCTGTAGCAAGCTC	57	n/a	n/a	79
664635	485	504	ACTCAGGAGTCGCGCGCTAG	64	n/a	n/a	80

Table 2

Inhibition of C9ORF72 Antisense Transcript by Antisense Oligonucleotides

Isis No.	Start	Stop	Sequence	LTS 01222	LTS 01221	C9ATS 3'-1	SEQ ID No.
129700	n/a	n/a	TAGTGCGGACCTACCCACGA	10	-5	n/a	41
661167	454	473	GGGGCCGGGGCCGGGGCCGG	69	52	n/a	42
	460	479					
	466	485					
661168	453	472	GGGCCGGGGCCGGGGCCGGG	60	36	n/a	43
	459	478					
	465	484					
661169	452	471	GGCCGGGGCCGGGGCCGGGG	65	37	n/a	44
	458	477					
	464	483					
661170	451	470	GCCGGGGCCGGGGCCGGGGC	66	18	n/a	45
	457	476					
	463	482					
661171	456	475	CCGGGGCCGGGGCCGGGGCC	77	56	n/a	46
	462	481					
661172	455	474	CGGGGCCGGGGCCGGGGCCG	65	40	n/a	47
	461	480					

Example 2: Antisense inhibition of C9ORF72 antisense transcript with oligonucleotides

5 Antisense oligonucleotides (ASOs) described in Example 1 were tested for inhibition of target transcript expression *in vitro* using a cell line in which a CMV promoter was installed to drive the expression of the endogenous C9ORF72 antisense gene via CRISPR/Cas9 technology.

The targeting portion of a single guide RNA (sgRNA) of the sequence 5'-GACAAGGGTACGTAATCTGTC-3', designated herein as SEQ ID NO: 81, was designed to target
10 a site 1,020 base pairs downstream of the C9ORF72 hexanucleotide repeat. An NGG PAM motif is present at the 3' end of the target site. The targeting portion of the sgRNA was inserted into a dual-expression plasmid to generate the full-length sgRNA of the sequence: 5'-GACAAGGGTACGTAATCTGTCTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCC

GTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT-3', designated herein as SEQ ID NO: 82 and Cas9 nuclease.

The donor plasmid, containing a 922 base pair 5' homology arm, reverse CMV (CMVr), and 988 base pair 3' homology arm, was generated in pCR4 (Life Technologies) backbone plasmid by Gibson Assembly. The homology arm sequences were designed to center around the CRISPR/Cas9 cleavage site and were constructed using PCR primers: 5'-TAGTCCTGCAGGTTTAAACGAATTCGTGAGTGAGGAGGCGGCA-3', forward primer, SEQ ID NO: 27, and 5'-AGCAGAGCTCAGATTACGTACCCTTGTTGTGAACAAC-3', reverse primer, SEQ ID NO: 28, for the 5' arm; and 5'-CAATGTCAACGTCTGGCATTACTTCTACTTTTG-3', forward primer, SEQ ID NO: 29, and 5'-TAGGGCGAATTGAATTTAGCGGCCGCACTGGCAGGATCATAGC-3', reverse primer, SEQ ID NO: 30, for the 3' arm. The CMVr sequence was amplified from pCDNA3.1 using PCR primers: 5'-TACGTAATCTGAGCTCTGCTTATATAGACC-3', forward primer, SEQ ID NO: 31, and 5'-AATGCCAGACGTTGACATTGATTATTGACTAGTTATTAATAG-3', reverse primer, SEQ ID NO: 32.

Neuroblast SH-SY5Y cells (Sigma-Aldrich) were cultured in a 1:1 mixture of MEM:F-12 (Life Technologies) supplemented with 10% FBS, 25mM HEPES and Antibiotic-Antimycotic. C9orf72 CRISPR/Cas9 activity was assessed by measuring indel frequency using SURVEYOR mutation detection assay (Integrated DNA Technologies) with forward primer: 5'-GTTAGGCTCTGGGAGAGTAGTTG-3', SEQ ID NO: 33, and reverse primer: 5'-CCTGGAGCAGGTAAATGCTGG-3', SEQ ID NO: 34. To generate SH-SY5Y cells expressing C9orf72 antisense transcript, SH-SY5Y cells were transfected with a plasmid expressing C9ORF72 CRISPR sgRNA and Cas9 and with a CMVr donor plasmid. Furthermore, the cells were co-transfected with a plasmid expressing EGFP, then single-cell sorted by FACS into 96-well plates. RT-qPCR was performed to screen for increased C9ORF72 antisense RNA. Positive clones were isolated and validated by PCR using primers inside CMVr and outside the 5' and 3' arms, respectively. Amplicons were further validated by sequencing to confirm on-target insertion of CMVr. Confirmation of single or double allele targeting was obtained by PCR with primers used in the SURVEYOR assay. Sequencing showed that the C9ORF72 antisense transcript contains two full hexanucleotide repeats.

The engineered SH-SY5Y cells were plated at 30,000 cells per well and electroporated at 140V with 10 μ M ASO. 24 hours later cells were lysed. Strand specific RT-qPCR was performed on

the isolated RNA, as described in Example 1, using the primer probe set LTS01221. The resulting normalized C9ORF72 antisense transcript levels were then compared to the normalized values for C9ORF72 antisense transcript expression in control cells that were transfected with neither the C9ORF72 antisense plasmid nor an ASO. The results are shown in the table below as percent inhibition of C9ORF72 antisense transcript expression relative to the control cells. A result of 0% inhibition indicates that the C9ORF72 antisense transcript levels were equal to that of control cells that were not transfected with an ASO. A negative value for % inhibition indicates that the C9ORF72 antisense transcript levels were higher than that of control cells that were not transfected with an ASO. The results show that many ASOs inhibited human C9ORF72 antisense transcript expression.

Table 3
Inhibition of C9ORF72 Antisense Transcript by Antisense Oligonucleotides

Isis No.	Inhibition (%)	SEQ ID No.
129700	-2	41
664570	37	48
664571	27	49
664572	66	50
664573	75	51
664574	59	52
664575	70	53
664576	75	54
664577	59	55
664578	65	56
664579	28	57
664580	34	58
664581	40	59
664582	50	60
664583	32	61
664584	48	62
664585	56	63
664586	66	64
664587	77	65
664588	64	66
664589	61	67
664590	59	68
664591	60	69
664592	62	70

664593	69	71
664594	67	72
664596	41	73
664599	26	74
664600	50	75
664611	68	76
664613	58	77
664623	3	78
664629	76	79
664635	60	80

Example 3: Dose dependent inhibition of C9ORF72 antisense transcript with oligonucleotides

Isis Numbers 664573, 664575, 664576, 664587, 664593, 664611, and 664629 (see Example 1) were tested for dose dependent inhibition of C9ORF72 antisense transcript expression *in vitro*.

5 The engineered SH-SY5Y cells described in Example 2 were cultured at 30,000 cells per well and electroporated at 140 V with an oligonucleotide at a concentration listed in the tables below or they received no ASO as a control. 24 hours after electroporation, cells were lysed and total RNA was isolated and analyzed by RT-qPCR using primer probe set LTS01221. The results are shown below for each antisense oligonucleotide concentration, and half maximal inhibitory concentrations were
 10 calculated using Prism software (Graphpad).

Table 4
Dose Dependent C9ORF72 Antisense Transcript Inhibition

Isis No.	Concentration (μM)	% Inhibition	IC ₅₀ (μM)
664573	0.625	-14	3.58
	1.25	8	
	2.5	35	
	5	48	
	10	66	
	20	69	
664575	0.625	-14	5.36
	1.25	7	
	2.5	17	
	5	41	
	10	53	
	20	72	

664576	0.625	-4	2.60
	1.25	4	
	2.5	57	
	5	51	
	10	61	
	20	78	
664587	0.625	1	2.34
	1.25	24	
	2.5	43	
	5	65	
	10	61	
	20	67	
664593	0.625	11	2.18
	1.25	27	
	2.5	44	
	5	56	
	10	71	
	20	67	
664611	0.625	13	2.49
	1.25	25	
	2.5	40	
	5	54	
	10	62	
	20	71	

Table 5**Dose Dependent C9ORF72 Antisense Transcript Inhibition**

Isis No.	Dose (nM)	% Inhibition	IC ₅₀ (μM)
664629	0.625	13	2.63
	1.25	28	
	2.5	36	
	5	49	
	10	64	
	20	68	

Example 4: Effect of antisense oligonucleotides targeting C9ORF72 antisense transcript on RNA foci

Antisense oligonucleotides targeting the C9ORF72 antisense transcript hexanucleotide repeat, listed in the table below, were tested for their effects on C9ORF72 antisense foci in C9ORF72 ALS/FTD patient fibroblast lines. Each nucleoside of the antisense oligonucleotides in the table below is modified with a 2'-MOE substitution. All of the cytosines are 5-methylcytosines, and all of the internucleoside linkages are phosphorothioate linkages.

Table 6

Fully modified antisense oligonucleotides targeting the antisense transcript of C9ORF72

Isis No.	Sequence	SEQ ID No.
663000	GGGGCCGGGGCCGGGGCCGG	42
663001	GGGCCGGGGCCGGGGCCGGG	43
663002	GGCCGGGGCCGGGGCCGGGG	44
663003	GCCGGGGCCGGGGCCGGGGC	45
663004	CCGGGGCCGGGGCCGGGGCC	46
663005	CGGGGCCGGGGCCGGGGCCG	47

10

C9ORF72 antisense foci were visualized using fluorescent *in situ* hybridization with a fluorescently labeled Locked Nucleic Acid (LNA) probe targeting the hexanucleotide repeat containing C9ORF72 antisense transcript (Exiqon, Inc. Woburn MA). The sequence of the probe is presented in the table below. The probe was labeled with fluorescent 5' TYE-563. A 5' TYE-563-labeled fluorescent probe targeting CUG repeats is used as a negative control.

15

Table 7

LNA probes to the C9ORF72 antisense transcript containing the hexanucleotide repeat

Target	Description of probe	Sequence	SEQ ID NO
GGCCCC Repeat of the Antisense Transcript	Fluorescent LNA Probe	TYE563- GGGGCCGGGGCCGGGG	83
CUG Repeat	Fluorescent LNA Probe	TYE563- CAGCAGCAGCAGCAGCAGC	84

20

All hybridization steps were performed under RNase-free conditions. Patient fibroblast cells were plated into chamber slides. 24 hours later, they were washed in PBS and transfected with 25 nM of an Isis antisense oligonucleotide in the table below or a negative control ASO that does not target any C9ORF72 RNA using 1 μ l/ml Cytofectin transfection reagent (Genlantis, San Diego, Cat# T610001). Cells were incubated for 4 hours at 37°C and 5% CO₂, before the medium was replaced with Dulbecco's modified Eagle medium (DMEM) supplemented with 20% tetracycline-free FBS and 2% penicillin/streptomycin and 1% amphotericin B. 24 hours after transfection, the cells were fixed in 4% PFA, then immediately permeabilized in 0.2% Triton X-100 (Sigma Aldrich #T-8787) in PBS for 10 minutes, washed twice in PBS for 5 minutes, dehydrated with ethanol, and air dried. The slides were heated in 400 μ L hybridization buffer (50% deionized formamide, 2xSCC, 50 mM Sodium Phosphate, pH 7, and 10% dextran sulphate) at 66°C for 20-60 minutes under floating RNase-free coverslips in a chamber humidified with hybridization buffer. Probes were denatured at 80°C for 75 seconds and returned immediately to ice before diluting with hybridization buffer (40 nM final concentration). The incubating buffer was replaced with the probe-containing mix (400 μ L per slide), and slides were hybridized under floating coverslips for 12-16 hours in a sealed, light-protected chamber.

After hybridization, floating coverslips were removed and slides were washed at room temperature in 0.1% Tween-20/2X SCC for 5 minutes before being subjected to three 10-minute stringency washes in 0.1xSCC at 65°C. The slides were then dehydrated through ethanol and air dried.

Primary visualization for quantification and imaging of foci was performed at 100x magnification using a Nikon Eclipse Ti confocal microscope system equipped with a Nikon CFI Apo TIRF 100X Oil objective (NA 1.49). Most foci are intra-nuclear but are also occasionally found in the cytoplasm. Treatment with RNase A, but not DNase I, eliminated the C9ORF72 antisense foci, demonstrating that they are comprised primarily of RNA. The foci in the fibroblasts were counted, and the data is presented in the table below as the number of foci per positive cell and the number of foci per cell overall. (A positive cell is a cell that has at least one focus.) The data in the table below show that treatment with the antisense oligonucleotides targeting the antisense C9ORF72 transcript, listed in the table below, decreased both the number of cells with at least one focus and the number of foci within cells that still had at least one focus.

Table 8
Antisense C9ORF72 foci in patient fibroblasts

Isis No.	Foci per positive cell	Foci per cell
Negative control ASO	2.94	1.52
663000	1.67	0.05
663001	1.20	0.12
663002	1.58	0.19
663003	1.00	0.04
663004	2.17	0.68
663005	1.86	0.36

CLAIMS

What is claimed is:

1. A compound comprising a modified oligonucleotide consisting of 12-30 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 consecutive nucleobases of any of the nucleobases sequences of SEQ ID NOs 42-80.
2. The compound of claim 1, wherein the modified oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to a C9ORF72 antisense transcript.
3. The compound of claim 2, wherein the C9ORF72 antisense transcript has the nucleobase sequence of SEQ ID NO: 11.
4. The compound of any of claims 1-3, wherein the modified oligonucleotide is a single-stranded modified oligonucleotide.
5. The compound of any of claims 1-4, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.
6. The compound of any of claim 5, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.
7. The compound of claims 5 or 6, wherein the modified oligonucleotide comprises at least one phosphodiester linkage.
8. The compound of claim 6, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.
9. The compound of any of claims 1-8, wherein at least one nucleoside of the modified oligonucleotide comprises a modified nucleobase.
10. The compound of claim 9, wherein the modified nucleobase is a 5-methylcytosine.
11. The compound of any of claims 1-10, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar.
12. The compound of claim 11, wherein each nucleoside of the modified oligonucleotide comprises a modified sugar.

13. The compound of claims 11 or 12, wherein the modified sugar is a bicyclic sugar.
14. The compound of claim 13, wherein the bicyclic sugar comprises a chemical bridge between the 4' and 2' positions of the sugar, wherein the chemical bridge is selected from: 4'-CH(R)-O-2' and 4'-(CH₂)₂-O-2', wherein R is independently selected from H, C₁-C₆ alkyl, and C₁-C₆ alkoxy.
15. The compound of claim 14, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is methyl.
16. The compound of claim 14, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is H.
17. The compound of claim 14, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is -CH₂-O-CH₃.
18. The compound of claims 11 or 12, wherein the modified sugar comprises a 2'-O-methoxyethyl group.
19. The compound of any of claims 1-11 and 13-18, wherein the modified oligonucleotide is a gapmer.
20. The compound of claim 19, wherein the gapmer is a 5-10-5 MOE gapmer.
21. A composition comprising the compound of any preceding claim or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.
22. The composition of claim 21 further comprising a C9ORF72 sense transcript specific inhibitor.
23. The composition of claim 22, wherein the C9ORF72 sense transcript specific inhibitor is any of a nucleic acid, aptamer, antibody, peptide, or small molecule.
24. The composition of claim 23, wherein the nucleic acid is a single-stranded nucleic acid or a double-stranded nucleic acid.
25. The composition of claim 23, wherein the nucleic acid is a siRNA.
26. The composition of claim 22, wherein the C9ORF72 sense transcript inhibitor is an antisense compound.
27. The composition of claim 26, wherein the antisense compound is an antisense oligonucleotide.
28. The composition of claim 26, wherein the antisense compound is a modified oligonucleotide.
29. The composition of claim 28, wherein the modified oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least

- 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to a C9ORF72 sense transcript.
30. The compound of claim 29, wherein the C9ORF72 sense transcript has the nucleobase sequence of SEQ ID NO: 1-10.
 31. Use of the compound or composition of any preceding claim for the manufacture of a medicament for treating a neurodegenerative disease.
 32. A method comprising administering to an animal the compound or composition of any preceding claim.
 33. The method of claim 32, wherein the animal is a human.
 34. The method of claims 32 or 33, wherein the compound prevents, treats, ameliorates, or slows progression of at least one symptom of a C9ORF72 associated disease.
 35. The method of claim 34, wherein the at least one symptom is selected from among impaired motor function, difficulty with respiration, muscle weakness, fasciculation and cramping of muscles, difficulty in projecting the voice, shortness of breath, difficulty in breathing and swallowing, inappropriate social behavior, lack of empathy, distractibility, changes in food preference, agitation, blunted emotions, neglect of personal hygiene, repetitive or compulsive behavior, and decreased energy and motivation.
 36. The method of claim 34, wherein the C9ORF72 associated disease is amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, or olivopontocerebellar degeneration (OPCD).
 37. The method of claim 36, wherein the amyotrophic lateral sclerosis (ALS) is familial ALS.
 38. The method of claim 36, wherein the amyotrophic lateral sclerosis (ALS) is sporadic ALS.
 39. The method of any of claims 32-38, wherein the administering reduces C9ORF72 antisense transcript associated RAN translation products.
 40. The method of claim 39, wherein the C9ORF72 antisense transcript associated RAN translation products are any of poly-(proline-alanine), poly-(proline-arginine), and poly-(proline-glycine).
 41. The method of any of claims 32-40, wherein the administering reduces C9ORF72 antisense foci.
 42. The method of any of claims 32-40, wherein the administering reduces C9ORF72 sense foci.
 43. The method of any of claims 32-42, wherein the administering is parenteral administration.

44. The method of claim 43, wherein the parenteral administration is any of injection or infusion.
45. The method of claim 44, wherein the parenteral administration is directly into the central nervous system (CNS).
46. The method of claims 43 or 44, wherein the parenteral administration is any of intrathecal administration or intracerebroventricular administration.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/26218

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C12N 15/11, A61K 48/00, C07H 21/04 (2015.01)
 CPC - A61K 38/00, C12N 2310/315, C12N 15/113, C12N 2310/321, C12N 2310/14
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 IPC(8)- C12N 15/11, A61K 48/00, C07H 21/04 (2015.01)
 CPC- A61K 38/00, C12N 2310/315, C12N 15/113, C12N 2310/321, C12N 2310/14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 USPC- 514/44A, 514/44R, 536/24.5

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google Scholar: C9ORF72, antisense, transcript, C9ORF72, GGGGCC repeat, GGGGCCGGGGCCGGGGCCGG, antisense, silencing
 GenCore 6.4.1: SEQ ID NO: 42, 11

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim, No.
X	WO 2014/062691 A2 (BENNETT, et al.) 24 April 2014 (24.04.2014) pg 13, ln 20-27; pg 61, ln 10-18, SEQ ID NO 2; nucleotides 153-923, 95.7% identity to SEQ ID NO:11; pg 61, Table 16, SEQ ID NO:340, 100 % identity to SEQ ID NO:42	1, 2, 4
A	NCBI Reference AC255463. Homo sapiens chromosome 9 clone 174779_ABC12_000049116500_D6, complete sequence. (16 July 2014) [Retrieved from the Internet 25 September 2015: <http://www.ncbi.nlm.nih.gov/nuccore/AC255463>]; nucleobases 13623-14403, 97.4% identity	3

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 23 September 2015 (23.09.2015)	Date of mailing of the international search report 23 OCT 2015
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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/26218

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 5-46
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-4, directed to a modified oligonucleotide. The modified oligonucleotide will be searched to the extent that it encompasses nucleotide sequence SEQ ID NO: 42. It is believed that claims 1-4 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass nucleotide sequence SEQ ID NO: 42. Additional nucleotide sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected nucleotide sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a modified oligonucleotide of SEQ ID NO:50, i.e. claims 1-4.

***** See Supplemental Sheet to continue *****

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.:
1-4, restricted to SEQ ID NO: 42.

- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
 - The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 - No protest accompanied the payment of additional search fees.

In Continuation of Box III. Observations where unity of invention is lacking:

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The special technical feature of each invention of Group I+ is a specific nucleotide sequence recited therein, because no significant structural similarities can readily be ascertained among the sequences, and further because SEQ ID NO:42 was known in the art at the time of the invention (please see US 2006/0286575 A1 to Farrell, et al.; SEQ ID NO: 1, nucleotides 1-20, 100% identity).

Common Technical Features

The inventions of Group I+ share the technical feature of a modified oligonucleotide consisting of 12-30 linked nucleosides. However, this shared technical feature does not represent a contribution over prior art as being anticipated by WO 2013/162363 A1 to De Visser, et al. (31 October 2013) (hereinafter "De Visser").

De Visser discloses a modified oligonucleotide for targeting a (GGGGCC)_n repeat and consisting of 12-30 linked nucleosides (pg 82, SEQ ID NO: 210, GGCQCC GGCQCC GGCQCC; pg 47, ln 1-5, "Most preferred oligonucleotide consists of 2'-O-methyl phosphorothioate RNA, has its base sequence that consists of base sequence SEQ ID NO: 210 and has a length of 18 nucleotides"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

The inventions of Group I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.