(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 February 2016 (04.02.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/019063 A1

(51) International Patent Classification:
C07H 21/02 (2006.01) *C12N 15/113* (2010.01)
A61K 31/7115 (2006.01)

(21) International Application Number:
PCT/US2015/042740

(22) International Filing Date:
29 July 2015 (29.07.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/030,596 29 July 2014 (29.07.2014) US

(71) Applicant: **ISIS PHARMACEUTICALS, INC.** [US/US]; 2855 Gazelle Court, Carlsbad, CA 92010 (US).

(72) Inventor: **RIGO, Frank**; 2855 Gazelle Ct., Carlsbad, CA 92010 (US).

(74) Agents: **GINGRICH, Brenden** et al.; Knobbe Martens LLP, 2040 Main Street, 14th Floor, Irvine, CA 92614 (US).

(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))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2016/019063 A1

(54) Title: COMPOSITIONS AND METHODS FOR MODULATING TAU EXPRESSION

(57) Abstract: Disclosed herein are methods for reducing expression of Tau mRNA and protein in an animal with Tau antisense compounds. Also disclosed are methods for modulating splicing of Tau mRNA in an animal with Tau antisense compounds. Such methods are useful to treat, prevent, or ameliorate neurodegenerative diseases in an individual in need thereof. Examples of neurodegenerative diseases that can be treated, prevented, and ameliorated with the administration Tau antisense oligonucleotides include Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy, Chronic Traumatic Encephalopathy, Epilepsy, and Dravet's Syndrome.

COMPOSITIONS AND METHODS FOR MODULATING TAU EXPRESSION

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 BIOL0257WOSEQ_ST25.txt, created July 20, 2015, which is 440 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD

Provided are methods for treating, preventing, or ameliorating neurodegenerative diseases, including tauopathies, Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), Epilepsy, and Dravet's Syndrome by inhibiting expression of Tau or modulating the splicing of Tau in an animal. Certain embodiments are directed to methods, compounds and compositions for treating, preventing or ameliorating a seizure disorder by inhibiting expression of Tau or modulating the splicing of Tau in an animal.

BACKGROUND

The primary function of Tau is to bind to and stabilize microtubules, which are important structural components of the cytoskeleton involved in mitosis, cytokinesis, and vesicular transport. Tau is found in multiple tissues, but is particularly abundant in axons of neurons.

Serine-threonine directed phosphorylation regulates the microtubule binding ability of Tau. Hyperphosphorylation promotes detachment of Tau from microtubules. Other post translational modifications of Tau have been described; however the significance of these is unclear. Phosphorylation of Tau is also developmentally regulated with higher phosphorylation in fetal tissues and much lower phosphorylation in the adult. One characteristic of neurodegenerative disorders is aberrantly increased Tau phosphorylation.

The microtubule network is involved in many important processes within the cell including structural integrity needed for maintaining morphology of cells and operating transport machinery. Since binding of Tau to microtubules stabilizes microtubules, Tau is likely to be a key mediator of some of these processes and disruption of normal Tau in neurodegenerative diseases may disrupt some of these key cellular processes.

One of the early indicators that Tau may be important in neurodegenerative syndromes was the recognition that Tau is a key component of neurofibrillary inclusions in Alzheimer's disease. In fact,

neurofibrillary inclusions are aggregates of hyperphosphorylated Tau protein. Along with amyloid beta containing plaques, neurofibrillary inclusions are a hallmark of Alzheimer's disease and correlate significantly with cognitive impairment. 95% of Tau accumulations in AD are found in neuronal processes and is termed neuritic dystrophy. The process(es) whereby this microtubule associated protein becomes disengaged from microtubules and forms accumulations of proteins and how this relates to neuronal toxicity is not well understood.

Neuronal Tau inclusions are a pathological characteristic of not only Alzheimer's disease, but also a subset of Frontotemporal dementia (FTD), PSP, and CBD. The link between Tau and neurodegeneration was solidified by the discovery that mutations in the Tau gene cause a subset of FTD. These genetic data have also highlighted the importance of the 3R:4R ratio of Tau. Many of the Tau mutations that cause FTD lead to a change in Tau splicing which leads to preferential inclusion of exon 10, and thus to increased 4R Tau. The overall Tau levels are normal. Whether the Tau isoform change or the amino acid change or both cause neurodegeneration remains unknown. Recent data suggest that PSP may also be associated with an increased 4R:3R Tau ratio and thus may be amenable to a similar splicing strategy.

To help understand the influence of Tau ratios on neurodegeneration, a mouse model based on one of the splicing Tau mutations (N279K) has been generated using a minigene that includes the Tau promoter and the flanking intronic sequences of exon 10. As in humans, these mice demonstrate increased levels of 4R Tau compared with transgenics expressing WT Tau and develop behavioral and motor abnormalities as well as accumulations of aggregated Tau in the brain and spinal cord.

The protein "Tau" has been associated with multiple diseases of the brain including Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal ganglionic degeneration, dementia pugilistica, parkinsonism linked to chromosome, Lytico-Bodig disease, tangle-predominant dementia, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, Pick's disease, argyrophilic grain disease, corticobasal degeneration or frontotemporal lobar degeneration and others. Tau-associated disorders such as AD are the most common cause of dementia in the elderly. AD affects an estimated 15 million people worldwide and 40% of the population above 85 years of age. AD is characterized by two pathological hallmarks: Tau neurofibrillary inclusions (NFT) and amyloid-β (Aβ) plaques.

In seizure disorders, the brain's electrical activity is periodically disturbed, resulting in some degree of temporary brain dysfunction. Normal brain function requires an orderly, organized, coordinated discharge of electrical impulses. Electrical impulses enable the brain to communicate with the spinal cord, nerves, and muscles as well as within itself. Seizures may result when the brain's electrical activity is disrupted. There are

two basic types of seizures; epileptic and nonepileptic. Epileptic seizures have no apparent cause or trigger and occur repeatedly. Nonepileptic seizures are triggered orprovoked by a disorder or another condition that irritates the brain. Certain mental disorders can cause seizure symptoms referred to as psychogenic nonepileptic seizures.

Alzheimer's Disease(AD) is known to be a clinical risk factor for late onset seizures. Multiple AD mouse models recapitulate this increased seizure susceptibility. Within the last 5 years, many of these AD models have been studied in the setting of mouse tau knockout (tau-/-). Increased seizure susceptibility was ameliorated in these amyloid-depositing tau knockout lines. Further, tau-/- alone interestingly appeared to be protective against chemically induced seizures.

Anticonvulsants represent the common treatment regime for seizues. However, anticonvulsants are ineffective in a significant percent of people with a seizure disorder and for these individuals, surgery is the only option. Amidst the lack of available treatments for seizure disorders and neurodegenerative diseases, certain methods of the present embodiments provide methods for treating, preventing or ameliorating a seizure disorder and neurodegenerative diseases by inhibiting expression of Tau or modulating the splicing of Tau in an animal.

SUMMARY

Provided herein are methods for modulating levels of Tau transcript (pre-mRNA and/or mRNA) and protein in cells, tissues, and animals. Also provided herein are methods for modulating splicing of Tau mRNA in cells, tissues, and animals. Also provided herein are methods for modulating the expression product of a Tau mRNA in cells, tissues, and animals.

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 human. In certain embodiments, Tau mRNA levels are reduced. In certain embodiments, Tau protein levels are reduced. In certain embodiments, splicing of Tau mRNA is modulated. In certain embodiments, the expression product of a Tau mRNA is modulated. In certain embodiments, hyperphosphorylated Tau is reduced. Such reduction and modulation can occur in a time-dependent manner or in a dose-dependent manner.

Several embodiments are drawn to methods of reducing or decreasing seizures in a subject. In certain embodiments, methods are provided for reducing the risk for seizure in a subject. In certain embodiments, the seizures are related to neurodegenerative disorders. In certain embodiments, the neurodegenerative disorder is a tau-associated disorder. In certain embodiments, the tau-associated disorder or neurodegenerative disorder is Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal ganglionic degeneration, dementia pugilistica, parkinsonism linked to chromosome, Lytico¬Bodig disease,

tangle-predominant dementia, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, Pick's disease, argyrophilic grain disease, corticobasal degeneration or frontotemporal lobar degeneration. Certain embodiments are drawn to a method of decreasing seizures in a subject.

Also provided are methods useful for preventing, treating, and ameliorating diseases, disorders, and conditions associated with Tau. In certain embodiments, such diseases, disorders, and conditions associated with Tau are neurodegenerative diseases. In certain embodiments, the neurodegenerative disease is any of Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy, Chronic Traumatic Encephalopathy, Epilepsy, or Dravet's Syndrome. In certain embodiments, one or more symptoms of a neurodegenerative disease is ameliorated, prevented, or delayed (progression slowed). In certain embodiments, the symptom is memory loss, anxiety, or loss of motor function. In certain embodiments, neurodegenerative function is improved. In certain embodiments, neurofibrillary inclusions are reduced.

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

In certain embodiments, methods of treatment include administering a Tau antisense compound to an individual in need thereof. The antisense compound may inhibit expression of Tau or modulate splicing of Tau. In certain embodiments, the antisense compound is an antisense oligonucleotide. In certain embodiments, the single-stranded antisense oligonucleotide is complementary to a Tau nucleic acid.

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

Embodiment 1. An antisense compound comprising a modified oligonucleotide consisting of 10-30 linked nucleosides and having a nucleobase sequence complementary to an intron/exon junction or an exon/intron junction or a splice modulation site of a Tau transcript.

Embodiment 2. The antisense compound of embodiment 1, wherein the antisense compound is single-stranded.

Embodiment 3. The antisense compound of embodiment 1, wherein the antisense compound is double-stranded.

Embodiment 4. The antisense compound of any of embodiments 1-3, wherein the antisense compound comprises at least one conjugate.

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

Embodiment 6. The antisense compound of embodiment 5, wherein the modified oligonucleotide comprises at least one modified nucleoside comprising a modified sugar.

Embodiment 7. The antisense compound of embodiment 6, wherein the at least one modified sugar is selected from among a bicyclic sugar, a non-bicyclic 2'-modifed sugar, and a sugar surrogate.

Embodiment 8. The antisense compound of embodiment 7, wherein at least one modified nucleoside is a 2'-MOE modified nucleoside.

Embodiment 9. The antisense compound of embodiment 7, wherein at least one modified nucleoside is a morpholino nucleoside.

Embodiment 10. The antisense compound of any of embodiments 5-9, wherein essentially each nucleoside of the modified oligonucleotide is modified.

Embodiment 11. The antisense compound of any of embodiments 5-9, wherein each nucleoside of the modified oligonucleotide is modified.

Embodiment 12. The antisense compound of any of embodiments 5-11, wherein each modified nucleoside has the same modification.

Embodiment 13. The antisense compound of any of embodiments 5-11, wherein at least two modified nucleoside have different modifications from one another.

Embodiment 14. The antisense compound of any of embodiments 1-13, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

Embodiment 15. The antisense compound of embodiment 14, wherein each internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.

Embodiment 16. The antisense compound of embodiment 14 or 15, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 17. The antisense compound of any of embodiments 1-16, wherein the modified oligonucleotide consists of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 linked nucleosides.

Embodiment 18. The antisense compound of any of embodiments 1-17, wherein the modified oligonucleotide is at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% complementary to an equal length portion of a human Tau nucleic acid.

Embodiment 19. The antisense compound of any of embodiments 1-17, wherein the modified oligonucleotide is 100% complementary to an equal length portion of a human Tau nucleic acid.

Embodiment 20. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon -1/intron -1 of the Tau transcript.

Embodiment 21. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron -1/exon 1 of the Tau transcript.

Embodiment 22. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 1/intron 1 of the Tau transcript.

Embodiment 23. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 1/exon 2 of the Tau transcript.

Embodiment 24. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 2/intron 2 of the Tau transcript.

Embodiment 25. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 2/exon 3 of the Tau transcript.

Embodiment 26. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 3/intron 3 of the Tau transcript.

Embodiment 27. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 3/exon 4 of the Tau transcript.

Embodiment 28. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 4/intron 4 of the Tau transcript.

Embodiment 29. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 4/exon 5 of the Tau transcript.

Embodiment 30. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 5/intron 5 of the Tau transcript.

Embodiment 31. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 5/exon 6 of the Tau transcript.

Embodiment 32. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 6/intron 6 of the Tau transcript.

Embodiment 33. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 6/exon 7 of the Tau transcript.

Embodiment 34. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 7/intron 7 of the Tau transcript.

Embodiment 35. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 7/exon 8 of the Tau transcript.

Embodiment 36. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 8/intron 8 of the Tau transcript.

Embodiment 37. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 8/exon 9 of the Tau transcript.

Embodiment 38. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 9/intron 9 of the Tau transcript.

Embodiment 39. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 9/exon 10 of the Tau transcript.

Embodiment 40. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 10/intron 10 of the Tau transcript.

Embodiment 41. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 10/exon 11 of the Tau transcript.

Embodiment 42. The antisense compound of any of embodiments 1-19, wherein the modified

oligonucleotide is complementary to the junction of exon 11/intron 11 of the Tau transcript.

Embodiment 43. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 11/exon 12 of the Tau transcript.

Embodiment 44. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 12/intron 12 of the Tau transcript.

Embodiment 45. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of intron 12/exon 13 of the Tau transcript.

Embodiment 46. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to the junction of exon 13/intron 13 of the Tau transcript.

Embodiment 47. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to a splice modulation site within intron -1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 of the Tau transcript.

Embodiment 48. The antisense compound of any of embodiments 1-19, wherein the modified oligonucleotide is complementary to a splice modulation site within exon -1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 of the Tau transcript.

Embodiment 49. The antisense compound of any of embodiments 1-48, wherein the Tau transcript is a mouse Tau transcript.

Embodiment 50. The antisense compound of any of embodiments 1-48, wherein the Tau transcript is a human Tau transcript.

Embodiment 51. The antisense compound of any of embodiments 1-49, wherein the modified oligonucleotide has a nucleobase sequence comprising at least 8, 10, 12, 14, 16, 18, 20, or 22 contiguous nucleobases of a nucleobase sequence selected from among of any of the oligonucleotides described in the present disclosure.

Embodiment 52. A method comprising contacting a cell with an antisense compound according to any of embodiments 1-51.

Embodiment 53. The method of embodiment 52, wherein the cell is in vitro.

Embodiment 54. The method of embodiment 52, wherein the cell is in an animal.

Embodiment 55. A method for reducing the amount or activity of a Tau transcript in a cell comprising contacting the cell with an antisense compound of any of embodiments 1-51 and thereby reducing the amount or activity of the Tau transcript in the cell.

Embodiment 56. The method of embodiment 56, wherein the amount of Tau transcript is reduced.

Embodiment 57. A method of treating a Tau disorder in an animal comprising administering to the animal an antisense compound according to any of embodiments 1-51.

Embodiment 58. The method of embodiment 57, wherein the Tau disorder is selected from among: Tauopathy, Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear

Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), Epilepsy, or Dravet's Syndrome.

Embodiment 59. The method of embodiment 57 or 58, wherein the animal is a mouse.

Embodiment 60. The method of embodiment 57 or 58, wherein the animal is a human.

DETAILED DESCRIPTION

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

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

Treatment of neurodegenerative syndrome and seizures

A method of modifying neurodegenerative disease has been developed. Using the methods of the invention, it is now possible to alter the ratio of tau isoforms associated with multiple diseases of the brain. Advantageously, the invention provides a method of bypassing the blood brain barrier to specifically target the generation of certain tau isoforms in the central nervous system, may be administered for an extended period of time using proven technology, and has been demonstrated to provide widespread distribution of therapy throughout the brain and spinal cord where it is most efficient.

I. Method

The present invention provides a method of modifying a neurodegenerative syndrome in a subject by administering an antisense compound to the central nervous system. In certain embodiments, the antisense compound alters splicing of the nucleic acid encoding tau and decreases the amount of tau mRNA, pre-mRNA, and/or tau protein the central nervous system of the subject.

(a) subject

According to the invention, the subject may be any subject that expresses tau. In some embodiments, a subject is a rodent, a human, a livestock animal, a companion animal, or a zoological animal. In one embodiment, the subject may be a rodent, e.g. a mouse, a rat, a guinea pig, etc. In another embodiment, the subject may be a livestock animal. Non-limiting examples of suitable livestock animals may include pigs, cows, horses, goats, sheep, llamas and alpacas. In still another embodiment, the subject may be a companion animal. Non-limiting examples of companion animals may include pets such as dogs, cats, rabbits, and birds. In yet another embodiment, the subject may be a zoological animal. As used herein, a "zoological animal" refers to an animal that may be found in a zoo. Such animals may include non-human primates, large cats, wolves, and bears. In an exemplary embodiment, the subject may be a human.

The subject may be suffering from a neurodegenerative syndrome or may be at risk of developing a neurodegenerative syndrome. In some embodiments, the subject may be suffering from a neurodegenerative syndrome. In other embodiments, the subject may be at risk of developing a neurodegenerative syndrome. Neurodegenerative syndromes are as described further below.

(b) neurodegenerative syndrome

The method of the invention comprises modifying a neurodegenerative syndrome. In some embodiments, a neurodegenerative syndrome may be any neurodegenerative syndrome associated with tau. Non limiting examples of a neurodegenerative disorder associated with tau may include Alzheimer's disease, progressive supranuclear palsy, dementia pugilistica, frontotemporal dementia, parkinsonism linked to chromosome, Lytico-Bodig disease, tangle-predominant dementia, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, Pick's disease, corticobasal ganglionic degeneration, argyrophilic grain disease, supranuclear palsy, corticobasal degeneration, frontotemporal dementia, or frontotemporal lobar degeneration. In some embodiments, the method of the invention comprises modifying frontotemporal dementia (FTD). In other embodiments, the method of the invention comprises modifying Alzheimer's disease (AD). In yet other embodiments, the method of the invention comprises modifying progressive supranuclear palsy. In other embodiments, the method of the invention comprises modifying corticobasalganglionic degeneration.

As used herein, the term "modifying a neurodegenerative syndrome" may refer to curing the neurodegenerative syndrome, slowing the course of development of the syndrome, reversing the course of the syndrome, or improving the behavioral phenotype of a subject having a neurodegenerative syndrome. In some embodiments, the method of the invention modifies a neurodegenerative syndrome by curing the neurodegenerative syndrome. In other embodiments, the method of the invention modifies a neurodegenerative syndrome by slowing the progression of the syndrome.

In yet other embodiments, the method of the invention modifies a neurodegenerative syndrome by improving the behavioral phenotype of a subject having a neurodegenerative syndrome. For instance, the

symptoms for subjects suffering from Alzheimer's disease may be the mild early symptoms associated with the neurodegenerative syndrome such as mild forgetfulness of recent events, activities, the names of familiar people or things, and the inability to solve simple math problems. The symptoms may also be the moderate symptoms associated with the neurodegenerative syndrome such as forgetting how to do simple tasks such as grooming, speaking, understanding, reading, or writing. Alternatively, the symptoms may be the severe symptoms associated with the neurodegenerative syndrome such as becoming anxious or aggressive, and wandering away from home. Subjects with AD may also have an increased risk of seizures. The symptoms for subjects suffering from progressive supranuclear palsy may include loss of balance, lunging forward when mobilizing, fast walking, bumping into objects or people, falls, changes in personality, general slowing of movement, visual symptoms, dementia (typically including loss of inhibition and ability to organize information), slurring of speech, difficulty swallowing, and difficulty moving the eyes, particularly in the vertical direction, poor eyelid function, contracture of the facial muscles, a backward tilt of the head with stiffening of the neck muscles, sleep disruption, urinary incontinence and constipation. The symptoms for subjects suffering from FTD may include personality changes, cognitive impairment, and motor symptoms. The symptoms for subjects suffering from corticobasalganglionic degeneration are similar to symptoms in patients suffering from FTD and Parkinson's disease and may include shaking, rigidity, slowness of movement and difficulty with walking and gait, cognitive and behavioural problems, dementia, sensory, sleep and emotional problems. In preferred embodiments, the method of the invention modifies a neurodegenerative syndrome by decreasing the risk of seizures.

Definitions

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

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

"Nonsense mediated decay" or "NMD" means any number of cellular mechanisms independent of RNase H or RISC that degrade mRNA or pre-mRNA. In certain embodiments, nonsense mediated decay eliminates and/or degrades mRNA transcripts that contain premature stop codons. In certain embodiments, nonsense mediated decay eliminates and/or degrades any form of aberrant mRNA and/or pre-mRNA transcripts.

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

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

"2'-OMethyl" means a 2'-OCH_3 modification at the 2' position of a furanosyl ring.

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

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

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

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

"About" means within ±7% of a value. For example, if it is stated, "the compounds affected at least about 70% inhibition of Tau", it is implied that the Tau levels are inhibited within a range of 63% and 77%.

"Acceptable safety profile" means a pattern of side effects that is within clinically acceptable limits.

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

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

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

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

"Agent" means an active substance that can provide a therapeutic benefit when administered to an animal. "First Agent" means a therapeutic compound described herein. For example, a first agent can be an antisense oligonucleotide targeting Tau. "Second agent" means a second therapeutic compound described herein (e.g. a second antisense oligonucleotide targeting Tau) and/or a non- Tau therapeutic compound.

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

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

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

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

"Antisense compound" means an oligomeric compound that is is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding. 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 or target protein levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

"Antisense mechanisms" are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing. Antisense mechanisms include, without limitation, RNase H mediated antisense; RNAi mechanisms, which utilize the RISC pathway and include, without limitation, siRNA, ssRNA, and microRNA mechanisms; and occupancy based mechanisms, including, without limitation uniform modified olionucleotides. Certain antisense compounds may act through more than one such mechanism and/or through additional mechanisms.

"Antisense oligonucleotide" (also "oligo") means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid resulting in at least one antisense activity.

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

"Bicyclic sugar" means a furanosyl 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 on the sugar ring.

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

"cEt" or "constrained ethyl" means a bicyclic nucleoside having a sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-$CH(CH_3)$-O-2'.

"Constrained ethyl nucleoside" (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-$CH(CH_3)$-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 nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

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

"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 administration in parallel or sequentially.

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

"Comply" means the adherence with a recommended therapy by an individual.

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

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

"Cure" means a method or course that restores health or a prescribed treatment for an illness.

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

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

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

"Dosage unit" means a form in which a pharmaceutical agent is provided, e.g. pill, tablet, or other dosage unit known in the art.

"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 two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired

dose requires a volume not easily accommodated by a single injection. In such embodiments, two or more injections may be used to achieve the desired dose. In certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual. In other 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.

"Dosing regimen" is a combination of doses designed to achieve one or more desired effects.

"Duration" means the period of time during which an activity or event continues. In certain embodiments, the duration of treatment is the period of time during which doses of a pharmaceutical agent are administered.

"Effective amount" in the context of modulating an activity or of treating or preventing a condition means the administration of that amount of active ingredient 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 will vary depending upon the health and physical condition of the subject to be treated, the taxonomic group of subjects to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors.

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

"Excitotoxicity" the pathological process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters.

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

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

"Fully modified motif" refers to an antisense compound comprising a contiguous sequence of nucleosides wherein essentially each nucleoside is a sugar modified nucleoside having uniform modification.

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

"Tau nucleic acid" or 'Tau DNA" means any nucleic acid encoding Tau. For example, in certain embodiments, a Tau nucleic acid includes, without limitation, any viral DNA sequence encoding a Tau genome or portion thereof, any RNA sequence transcribed from a DNA sequence including any mRNA sequence encoding a Tau protein.

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

"Identifying an animal having a Tau-related disease or disorder" means identifying an animal having been diagnosed with a Tau-related disease or disorder; or, identifying an animal having any symptom of Tau-related disease or disorder including, but not limited to a neurodegenerative disorder associated with Tau.

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

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

"Individual compliance" means adherence to a recommended or prescribed therapy by an individual.

"Induce", "inhibit", "potentiate", "elevate", "increase", "decrease" or the like, e.g., which denote quantitative differences between two states, refer to at least statistically significant differences between the two states. For example, "an amount effective to inhibit the activity or expression of Tau" means that the level of activity or expression of Tau in a treated sample will differ statistically significantly from the level of Tau activity or expression in untreated cells. Such terms are applied to, for example, levels of expression, and levels of activity.

"Inhibiting Tau" means reducing the level or expression of a Tau mRNA, DNA and/or protein . In certain embodiments, Tau is inhibited in the presence of an antisense compound targeting Tau, including an antisense oligonucleotide targeting Tau, as compared to expression of Tau mRNA, DNA and/or protein levels in the absence of a Tau antisense compound, such as an antisense oligonucleotide.

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

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

"Intraperitoneal administration" means administration through infusion or injection into the peritoneum.

"Intravenous administration" means administration into a vein.

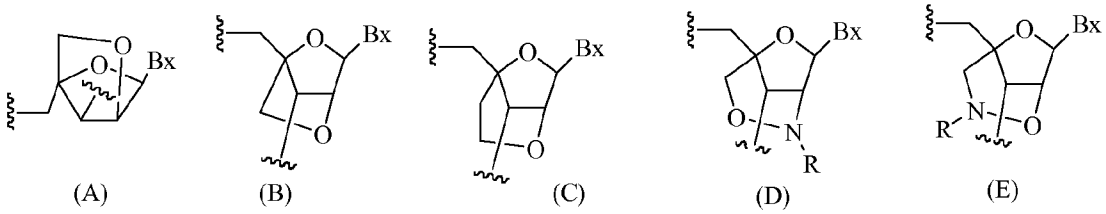
"Lengthened" antisense oligonucleotides are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein.

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

"Linked nucleosides" means adjacent nucleosides linked together by an internucleoside linkage.

"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'-CH_2-O-2') LNA , (B) β-D-Methyleneoxy (4'-CH_2-O-2') LNA , (C) Ethyleneoxy (4'-$(CH_2)_2$-O-2') LNA , (D) Aminooxy (4'-CH_2-O-N(R)-2') LNA and (E) Oxyamino (4'-CH_2-N(R)-O-2') LNA, as depicted below.



(A) (B) (C) (D) (E)

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 bicylic 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.

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

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

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

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

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

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

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

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

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

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

"Neurodegenerative disorder" means a chronic progressive neuropathy characterized by selective loss of neurons in motor, sensory, or cognitive systems. Neurodegenerative disorders include, but are not limited to, Tau-associated disorders.

"Neurofibrillary inclusion" means intrneuronal aggregates largely composed of insoluble hyperphosphorylated tau protein. In certain embodiments, neurofibrillary inclusions may be measured through various means including SPECT perfusion imaging, functional MRI, and PET scans. In certain embodiments, reduction of neurofibrillary inclusions may be inferred by improved scores on cognitive exams such as the Mini-Mental State Exam (MMSE) and the Alzheimer's Disease Assessment Scale Cognitive Behavior Section (ADAS-cog).

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

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

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

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

with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair.

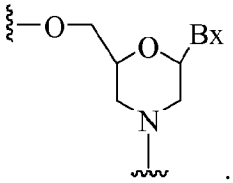
"Nucleobase sequence" means the order of contiguous nucleobases independent of any sugar, linkage, and/or nucleobase modification.

"Nucleoside" means a nucleobase linked to a sugar.

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

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 structure:



.

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 refered to herein as "modifed morpholinos."

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

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

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

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

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

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

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

"Pharmaceutically acceptable carrier" means a medium or diluent that does not interfere with the structure of the oligonucleotide. Certain such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject.

"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.

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

"Pharmaceutical composition" means a mixture of substances suitable for administering to a subject. For example, a pharmaceutical composition may comprise an antisense oligonucleotide and a sterile aqueous solution. In certain embodiments, a pharmaceutical composition shows activity in free uptake assay in certain cell lines.

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

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

"Prevention" or "preventing" refers to delaying or forestalling the onset or development of a condition or disease for a period of time from hours to days, preferably weeks to months.

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

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

"Recommended therapy" means a therapeutic regimen recommended by a medical professional for the treatment, amelioration, or prevention of a disease.

"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.

"Scrambled oligo" or "scrambled" or "ISIS 141923" is a 5-10-5 MOE gapmer with no known target having the sequence of SEQ ID NO: 11.

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

"Shortened" or "truncated" versions of antisense oligonucleotides Taught herein have one, two or more nucleosides deleted.

"Side effects" means physiological responses attributable to a treatment other than desired effects. In certain embodiments, side effects include, without limitation, injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

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

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

"Splice modulation site" refers to a site on the Tau pre-mRNA which when hybridized by an antisense oligonucleotide results in altered splicing of the Tau pre-mRNA resulting in an altered splice product. In certain embodiments, such altered splice product is less stable or results in reduced amount of tau protein. In certain embodiments, a splice modulation site comprises a region of Tau pre-mRNA containing an intronic splice silencer. In certain embodiments, a splice modulation site comprises a region of Tau pre-mRNA containing an intronic splice enhancer. In certain embodiments, a splice modulation site comprises a region of Tau pre-mRNA containing serine/arginine-rich splicing factor.

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

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

"Subcutaneous administration" means administration just below the skin.

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

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

"Target gene" refers to a gene encoding a target.

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

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

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

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

"Tau-associated disease" means any neurological or neurodegenerative disease associated with Tau. Non-limiting examples of Tau-associated disorders include Alzheimer's disease, progressive supranuclear palsy, dementia pugilistica, frontotemporal dementia, parkinsonism linked to chromosome, Lytico-Bodig disease, tangle-predominant dementia, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, Pick's disease, corticobasal ganglionic degeneration, argyrophilic grain disease, supranuclear palsy, corticobasal degeneration, frontotemporal dementia, or frontotemporal lobar degeneration.

"Tauopathy" means disorders characterized by a build-up of Tau protein in the brain.

"Tau-specific inhibitor" includes but is not limited to a "antisense compound" targeted to Tau.

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

"Treatment" refers to administering a composition to effect an alteration or improvement of the disease or condition.

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

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

"Validated target segment" is defined as at least an 8-nucleobase portion (i.e. 8 consecutive nucleobases) of a target region to which an active oligomeric compound is targeted.

"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 for methods of administering a Tau antisense compound targeting a Tau nucleic acid for the treatment of a Tau associated disease. In certain embodiments, the Tau nucleic acid is any of the sequences set forth in GENBANK Accession NT_010783.14 truncated from nucleotides 2624000 to 2761000 (incorporated herein as SEQ ID NO: 1); GENBANK Accession No. AK226139.1 (incorporated herein as SEQ ID NO: 2); GENBANK Accession No. NM_001123066.3 (incorporated herein as SEQ ID NO: 3); GENBANK Accession No. NM_001123067.3 (incorporated herein as SEQ ID NO: 4); GENBANK Accession No. NM_001203251.1 (incorporated herein as SEQ ID NO: 5); GENBANK Accession No. NM_001203252.1 (incorporated herein as SEQ ID NO: 6); GENBANK Accession No. NM_005910.5 (incorporated herein as SEQ ID NO: 7); GENBANK Accession No. NM_016834.4 (incorporated herein as SEQ ID NO: 8); GENBANK Accession No. NM_016835.4 (incorporated herein as SEQ ID NO: 9); or GENBANK Accession No. NM_016841.4 (incorporated herein as SEQ ID NO: 10).

A method of treating a Tau associated disease with antiense compounds has been developed. In certain embodiments, neurofibrillary inclusions are reduced. In certain embodiments, neurological function is improved. In certain embodiments, the antisense compounds reduce expression of Tau mRNA and protein. In certain embodiments, the antisense compounds alter the ratio of Tau isoforms. In certain embodiments, the splicing alteration is a decrease in 4R:3R Tau ratio in the central nervous system of the subject. In certain embodiments, the splicing alteration results in a normal 4R:3R Tau ratio. Advantageously, several embodiments provide methods of bypassing the blood brain barrier to specifically target Tau in the central nervous system, administer for an extended period of time, and achieve widespread distribution of therapy throughout the brain and spinal cord where it is most effective.

Certain embodiments provide methods for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with Tau in an individual in need thereof. Also contemplated are methods for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with Tau. Tau associated diseases, disorders, and conditions include neurodegenerative diseases. In certain embodiments, the neurodegenerative disease may be any of Alzheimer's Disease,

frontotemporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), epilepsy, Dravet's Syndrome, dementia pugilistica, parkinsonism linked to chromosome, Lytico-Bodig disease, tangle-predominant dementia, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, Pick's disease, argyrophilic grain disease, supranuclear palsy, corticobasal degeneration, or frontotemporal lobar degeneration.

Described herein are methods comprising administering a Tau antisense compound to an animal for treating a Tau associated disease and thereby reducing neurofibrillary inclusions.

Described herein are methods comprising administering a Tau antisense compound to an animal for treating a Tau associated disease and thereby improving neurological function.

Described herein are methods comprising: (i) identifying an animal having a Tau associated disease; and (ii) administering a Tau antisense compound and thereby reducing neurofibrillary inclusions.

Described herein are methods comprising: (i) identifying an animal having a Tau associated disease; and (ii) administering a Tau antisense compound and thereby improving neurological function.

In certain embodiments, the animal is a human.

In certain embodiments, the antisense compound comprises a single-stranded antisense oligonucleotide complementary to a Tau nucleic acid.

In certain embodiments, the Tau nucleic acid is any of SEQ ID NO: 1-10.

In certain embodiments, the antisense compounds for use in the methods may comprise a single-stranded antisense oligonucleotide comprising a nucleobase sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of SEQ ID NOs:1-10. In certain embodiments, the compound may comprise a single-stranded antisense oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NOs: 1-10.

In certain embodiments, the Tau associated disease is a neurodegenerative disease.

In certain embodiments, the neurodegenerative disease is selected from among Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), Epilepsy, or Dravet's Syndrome.

In certain embodiments, expression of Tau RNA or expression of Tau protein is reduced.

In certain embodiments, expression of the 4R isoform of Tau RNA or expression of the 4R isoform of Tau protein is reduced.

In certain embodiments, expression of the 3R isoform of Tau RNA or expression of the 3R isoform of Tau protein is increased.

In certain embodiments, expression of the 4R isoform of Tau RNA is reduced and expression of the 3R isoform of Tau RNA is increased.

In certain embodiments, expression of the 4R isoform of Tau protein is reduced and expression of the 3R isoform of Tau protein is increased.

In certain embodiments, the single-stranded antisense oligonucleotide comprises at least one modification.

In certain embodiments, the single-stranded antisense oligonucleotide is specifically hybridizable to a human Tau nucleic acid.

In certain embodiments, the single-stranded antisense oligonucleotide is at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% complementary to an equal length portion of a human Tau nucleic acid.

In certain embodiments, the single-stranded antisense oligonucleotide is 100% complementary to a human Tau nucleic acid.

In certain embodiments, the single-stranded antisense oligonucleotide comprises at least one modified internucleoside linkage.

In certain embodiments, each internucleoside linkage of the single-stranded antisense oligonucleotide is a modified internucleoside linkage.

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

In certain embodiments, the antisense oligonucleotide comprises at least one modified nucleoside.

In certain embodiments, the single-stranded antisense oligonucleotide comprises at least one modified nucleoside having a modified sugar.

In certain embodiments, the single-stranded antisense oligonucleotide comprises at least one modified nucleoside comprising a bicyclic sugar.

In certain embodiments, the bicyclic sugar comprises a 4' to 2' bridge selected from among: 4'-(CH2)n-O-2', wherein n is 1 or 2; and 4'-CH2-O-CH2-2'.

In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

In certain embodiments, the at least one modified nucleoside having a modified sugar comprises a non-bicyclic 2'-modified sugar moiety.

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

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

In certain embodiments, the at least one modified nucleoside having a modified sugar comprises a sugar surrogate.

In certain embodiments, the sugar surrogate is a morpholino.

In certain embodiments, the sugar surrogate is a peptide nucleic acid.

In certain embodiments, each nucleoside is modified.

In certain embodiments, the single-stranded antisense oligonucleotide comprises at least one modified nucleobase.

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

In certain embodiments, fully 2'-MOE modified antisense oligonucleotides targeted to a tau transcript are administered intrathecally to the CNS. Like the fully modified 2'-MOE oligonucleotides described in WO2010/148249, the fully modified 2'-MOE oliognucleotides described herein are expected to be well tolerated when administered to the CNS.

Antisense compounds

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

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

In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 10 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 12 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 12 to 22 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 14 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 14 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 15 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 15 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 16 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 16 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 17 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 17 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 18 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 18 to 21 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 18 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 20 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits, 14 to 30 linked subunits, 14 to 20 subunits, 15 to 30 subunits, 15 to 20 subunits, 16 to 30 subunits, 16 to 20 subunits, 17 to 30 subunits, 17 to 20 subunits, 18 to 30 subunits, 18 to 20 subunits, 18 to 21 subunits, 20 to 30 subunits, or 12 to 22 linked subunits, respectively. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 14 subunits in length. In certain embodiments, an antisense compound targeted to a Tau nucleic acid is 16

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

In certain embodiments, an antisense compound targeted to a Tau nucleic acid is a single stranded ribonucleic acid or deoxyribonucleic acid antisense oligonucleotide. Antisense oligonucleotides may target a specific, complementary, coding or non-coding, nucleic acid. Depending on the antisense oligonucleotide used, the binding of the oligonucleotide to its target nucleic acid sequence may or may not activate RNAse H. In some embodiments, the antisense oligonucleotide activates RNAse H, which degrades the target nucleic acid. The antisense oligonucleotides of several embodiments may be any length provided it binds selectively to the intended location. In general, the antisense oligonucleotide may be from 8, 10 or 12 nucleotides in length up to 20, 30, or 50 nucleotides in length.

In certain embodiments antisense oligonucleotides targeted to a Tau 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 Tau nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

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

It is possible to increase or decrease the length of an antisense compound, such as an antisense

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

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

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

Antisense Compound Motifs

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

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

In certain embodiments, the antisense compounds are uniform sugar-modified oligonucleotides. Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the

substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides. In certain embodiments, wings may include several modified sugar moieties, including, for example 2'-MOE. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides and 2'-deoxynucleosides.

Each distinct region may comprise uniform sugar moieties, variant, or alternating sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5'-wing, "Y" represents the length of the gap, and "Z" represents the length of the 3'-wing. "X" and "Z" may comprise uniform, variant, or alternating sugar moieties. In certain embodiments, "X" and "Y" may include one or more 2'-deoxynucleosides."Y" may comprise 2'-deoxynucleosides. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap is positioned immediately adjacent to each of the 5'-wing and the 3' wing. Thus, no intervening nucleotides exist between the 5'-wing and gap, or the gap and the 3'-wing. Any of the antisense compounds described herein can have a gapmer motif. In certain embodiments, "X" and "Z" are the same; in other embodiments they are different. In certain embodiments, Y is between 8 and 15 nucleosides. X, Y, or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, or more nucleosides. Thus, gapmers described herein include, but are not limited to, for 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, antisense compounds targeted to a Tau nucleic acid possess a 5-8-5 gapmer motif.

In certain embodiments, an antisense compound targeted to a Tau nucleic acid has a gap-narrowed motif. In certain embodiments, a gap-narrowed antisense oligonucleotide targeted to a Tau 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 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'-modified sugar moiety comprises a 2'-O-methylethyl group or a 2'-O-methyl group.

In certain embodiments, an antisense compound targeted to a Tau nucleic acid is uniformly modified. In certain embodiments, the antisense compound comprises 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides. In certain embodiments, each nucleoside is chemically modified. In certain embodiments,

the chemical modification comprises a non-bicyclic 2'-modified sugar moiety. In certain embodiments, the 2'-modified sugar moiety comprises a 2'-O-methoxyethyl group. In certain embodiments, the 2'-modified sugar moiety comprises a 2'-O-methyl group.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode Tau include, without limitation, the following: GENBANK Accession NT_010783.14 truncated from nucleotides 2624000 to 2761000 (incorporated herein as SEQ ID NO: 1); GENBANK Accession No. AK226139.1 (incorporated herein as SEQ ID NO: 2); GENBANK Accession No. NM_001123066.3 (incorporated herein as SEQ ID NO: 3); GENBANK Accession No. NM_001123067.3 (incorporated herein as SEQ ID NO: 4); GENBANK Accession No. NM_001203251.1 (incorporated herein as SEQ ID NO: 5); GENBANK Accession No. NM_001203252.1 (incorporated herein as SEQ ID NO: 6); GENBANK Accession No. NM_005910.5 (incorporated herein as SEQ ID NO: 7); GENBANK Accession No. NM_016834.4 (incorporated herein as SEQ ID NO: 8); GENBANK Accession No. NM_016835.4 (incorporated herein as SEQ ID NO: 9); or GENBANK Accession No. NM_016841.4 (incorporated herein as SEQ ID NO: 10).

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

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

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

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

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

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

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

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in Tau mRNA levels are indicative of inhibition of Tau expression. Reductions in levels of a Tau protein are also indicative of inhibition of target mRNA expression. In certain embodiments, reductions in the 4R isoform of Tau mRNA levels are indicative of modulation of Tau splicing. Reductions in levels of the 4R isoform of Tau protein are also indicative of modulation of Tau splicing. In certain embodiments, increases in the 3R isoform of Tau mRNA levels are indicative of modulation of Tau splicing. Increases in levels of the 3R isoform of Tau protein are also indicative of modulation of Tau splicing. Reduction in percent of cells staining positive for hyperphosphorylated Tau are indicative of inhibition of Tau expression or modulation of Tau splicing. Improvement in neurological function is indicative of inhibition of Tau expression or modulation of Tau splicing. Improved memory and motor function are indicative of inhibition of Tau expression or modulation of Tau splicing. Reduction of neurofibrillary inclusions is indicative of inhibition of Tau expression or modulation of Tau splicing.

Hybridization

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

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

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

Complementarity

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

Non-complementary nucleobases between an antisense compound and a Tau 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 Tau nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

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

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

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For

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

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

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

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

The antisense compounds provided also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least

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

Identity

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

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

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

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

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar.

Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

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

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

Modified Internucleoside Linkages

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

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

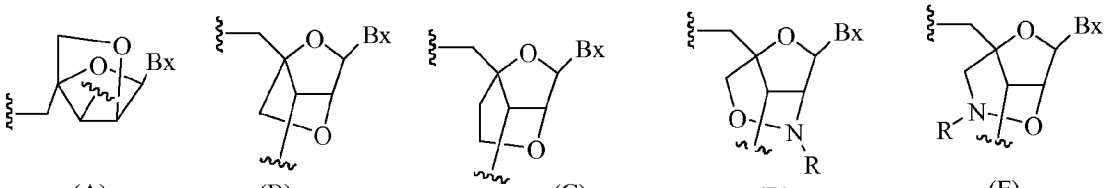
In certain embodiments, antisense compounds targeted to a Tau nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are interspersed throughout the antisense compound. In certain embodiments, the modified internucleoside linkages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

Modified Sugar Moieties

Oligomeric compounds provided herein may comprise one or more monomers, including a nucleoside or nucleotide, having a modified sugar moiety. For example, the furanosyl sugar ring of a nucleoside or nucleotide can be modified in a number of ways including, but not limited to, addition of a substituent group and bridging of two non-geminal ring atoms to form a Locked Nucleic Acid (LNA).

In certain embodiments, oligomeric compounds comprise one or more monomers having a bicyclic sugar. In certain embodiments, the monomer is an LNA. In certain such embodiments, LNAs include, but

are not limited to, (A) α-L-Methyleneoxy (4'-CH_2-O-2') LNA , (B) β-D-Methyleneoxy (4'-CH_2-O-2') LNA , (C) Ethyleneoxy (4'-$(CH_2)_2$-O-2') LNA , (D) Aminooxy (4'-CH_2-O-N(R)-2') LNA and (E) Oxyamino (4'-CH_2-N(R)-O-2') LNA, as depicted below.



(A) (B) (C) (D) (E)

In certain embodiments, 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.

In one embodiment, each of the bridges of the LNA compounds is, independently, -$[C(R_1)(R_2)]_n$-, -$[C(R_1)(R_2)]_n$-O-, -$C(R_1R_2)$-$N(R_1)$-O- or –$C(R_1R_2)$-O-$N(R_1)$-. In another embodiment, each of said bridges is, independently, 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'- wherein each R_1 is, independently, H, a protecting group or C_1-C_{12} alkyl.

Certain LNA's have been prepared and disclosed in the patent literature as well as in scientific literature (see for example: issued U.S. Patent Nos. 7,053,207; 6,268,490; 6,770,748; 6,794,499; 7,034,133; 6,525,191; 7,696,345; 7,569,575; 7,314,923; 7,217,805; and 7,084,125, hereby incorporated by reference herein in their entirety.

Also provided herein are LNAs in which the 2'-hydroxyl group is connected, to the 4' carbon atom of the ribosyl sugar ring, thereby forming a methyleneoxy (4'-CH_2-O-2') bridge to form the bicyclic sugar moiety (reviewed in Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8* 1-7; and Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; see also U.S. Patent: 6,670,461). Furthermore, the bridge can also be a methylene (-CH_2-) group connecting the 2' oxygen atom to the 4' carbon atom of the sugar ring, for which the term methyleneoxy (4'-CH_2-O-2') LNA is used. In the case of the bicylic sugar moiety having an ethylene bridging group in this position, the term ethyleneoxy (4'-CH_2CH_2-O-2') LNA is used (Singh et al., Chem. Commun., 1998, *4*, 455-456: Morita *et al., Bioorganic Medicinal Chemistry*, 2003, *11*, 2211-2226). Methyleneoxy (4'-CH_2-O-2') LNA and other bicyclic sugar analogs display very high duplex thermal stabilities with complementary DNA and RNA (Tm = +3 to +10 °C), stability towards 3'-exonucleolytic degradation and good solubility properties. Potent and nontoxic antisense oligonucleotides comprising LNAs have been described (Wahlestedt *et al., Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638).

An isomer of methyleneoxy (4'-CH_2-O-2') LNA that has also been discussed is α-L- methyleneoxy (4'-CH_2-O-2') LNA which has been shown to have superior stability against a 3'-exonuclease. The α-L-methyleneoxy (4'-CH_2-O-2') LNA's were incorporated into antisense gapmers and chimeras that showed potent antisense activity (Frieden *et al., Nucleic Acids Research,* 2003, *21*, 6365-6372).

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

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

As used herein, "bicyclic nucleoside" refers to a nucleoside comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic sugar moiety. In certain embodiments, the bridge connects the 2' carbon and another carbon of the sugar ring.

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

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

Many other bicyclic and tricyclic sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds as provided herein (*see, e.g.*, review article: Leumann, J. C, *Bioorganic & Medicinal Chemistry*, 2002, *10*, 841-854). Such ring systems can undergo various additional substitutions to further enhance their activity. Such ring systems can undergo various additional substitutions to enhance 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 unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C_1-C_{12} alkyl; substituted alkyl; alkenyl; alkynyl; alkaryl; aralkyl; O-alkaryl or O-aralkyl; SH; SCH_3; OCN; Cl; Br; CN; CF_3; OCF_3; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving pharmacokinetic properties; and a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain (*see, e.g.*, Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- *O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (*see, e.g.*, Martin, P., *Helv. Chim. Acta*, 1995, *78*, 486-504; Altmann et al., *Chimia*, 1996, *50*, 168-176; Altmann et al., *Biochem. Soc. Trans.*, 1996, *24*, 630-637; and Altmann et al., *Nucleosides Nucleotides*, 1997, *16*, 917-926).

As used herein, "2'-modified nucleoside" or "2'-substituted nucleoside" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position of a furanose ring other than H or OH. 2' modified nucleosides include, but are not limited to, nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, -OCF_3, O-$(CH_2)_2$-O-CH_3, 2'-O$(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-N$(R_m)(R_n)$, or O-CH_2-C(=O)-N$(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted

or unsubstituted C_1-C_{10} alkyl. 2'-modifed nucleosides may further comprise other modifications, for example, at other positions of the sugar and/or at the nucleobase.

As used herein, "2'-F" refers to modification of the 2' position of the furanosyl sugar ring to comprise a fluoro group.

As used herein, "2'-OMe" or "2'-OCH_3" or "2'-O-methyl" each refers to modification at the 2' position of the furanosyl sugar ring to comprise a -OCH_3 group.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA). Methods for the preparations of modified sugars are well known to those skilled in the art.

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

In certain embodiments, antisense compounds comprise one or more nucleosides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE.

Occupancy

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

In certain embodiments, antisense oligonucleotides do not activate RNAse H. In several aspects, antisense oligonucleotides that do not activate RNAse H are complementary to a nucleic acid sequence encoding Tau and disrupts the splicing of the nucleic acid encoding Tau to reduce the 4R:3R Tau ratio.

The antisense oligonucleotide of several embodiments may disrupt the splicing of the nucleic acid encoding Tau to reduce the 4R:3R Tau ratio. The splicing process is a series of reactions, mediated by splicing factors, which is carried out on RNA after transcription but before translation, in which the intron(s) are removed, and the exons joined together sequentially so that the protein may be translated. Each intron is defined by a 5' splice site, a 3' splice site, and a branch point situated there between. An antisense oligonucleotide may block these splice elements when the oligonucleotide either fully or partially overlaps the element, or binds to the pre-mRNA at a position sufficiently close to the element to disrupt the binding and function of the splicing factors which would ordinarily mediate the particular splicing reaction which occurs at that element. The antisense oligonucleotide may block a variety of different splice elements to carry out certain embodiments. For instance, the antisense oligonucleotide may block a mutated element, a cryptic element, or a native element; it may block a 5' splice site, a 3' splice site, or a branch point.

Methods of making antisense oligonucleotides which do not activate RNase H are known in the art. See, e.g., U.S. Pat. No. 5,149,797 incorporated herein by reference. Such antisense oligonucleotides may contain one or more structural modification which sterically hinders or prevents binding of RNase H to a duplex molecule comprising the oligonucleotide, but does not substantially hinder or disrupt duplex formation. Antisense oligonucleotides that do not activate RNAse H may include oligonucleotides wherein at least one, two or more of the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For instance, every other one of the internucleotide bridging phosphate residues may be a modified phosphate, contain a 2' loweralkyl moiety (e.g., C1-C4, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-propenyl, and isopropyl) or a combination thereof. In preferred embodiments, the antisense oligonucleotide of the invention that does not activate RNAse H, and disrupts the splicing of the nucleic acid encoding Tau to reduce the 4R:3R Tau ratio is a 2'-O-(2-methoxyethyl) (MOE)-modified antisense oligonucleotide.

Other methods of modifying an oligonucleotide to hinder binding of RNAse H may be found in P. Furdon et al., Nucleic Acids Res. 17, 9193-9204 (1989); S. Agrawal et al., Proc. Natl. Acad. Sci. USA 87, 1401-1405 (1990); C. Baker et al., Nucleic Acids Res. 18, 3537-3543 (1990); B. Sproat et al., Nucleic Acids Res. 17, 3373-3386 (1989); R. Walder and J. Walder, Proc. Natl. Acad. Sci. USA 85,5011-5015 (1988) the disclosures of all of which are incorporated herein, in their entirety, by reference.

Compositions and Methods for Formulating Pharmaceutical Compositions

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

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

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of

antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts. A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

In certain embodiments, an antisense oligonucleotide can include a physiologically and pharmaceutically acceptable salts thereof: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. Examples of such salts are (a) salts formed with cations such as sodium, potassium, NH4+, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and iodine.

Administration

Antisense oligonucleotides of certain embodiments may be administered to a subject by several different means. For instance, oligonucleotides may generally be administered parenterally, intraperitoneally, intravascularly, or intrapulmonarily in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. In a preferred embodiment, the oligonucleotide may be administered parenterally.

The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intrathecal, or intrasternal injection, or infusion techniques. Formulation of pharmaceutical compositions is discussed in, for example, Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (1975), and Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, NY. (1980).

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are useful in the preparation of injectables. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, and

polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

Delivery methods are preferably those that are effective to circumvent the blood-brain barrier and are effective to deliver agents to the central nervous system. For example, delivery methods may include the use of nanoparticles. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein.

Positively charged lipids such as N-[1-(2,3-dioleoyloxi)propyl)-N, N,N-trimethyl-amoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known in the art. See, e.q., U.S. Pat. Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.: 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.: 4,921,757 to Wheatley et al.; etc.

In one embodiment, the compounds provided herein may be administered in a bolus directly into the central nervous system. The compounds provided herein may be administered to the subject in a bolus once, or multiple times. In some preferred embodiments, the compounds provided herein may be administered once. In other preferred embodiments, the compounds provided herein may be administered multiple times. When administered multiple times, the compounds provided herein may be administered at regular intervals or at intervals that may vary during the treatment of a subject. In some embodiments, the compounds provided herein may be administered multiple times at intervals that may vary during the treatment of a subject. In some embodiments, the compounds provided herein may be administered multiple times at regular intervals.

In another preferred embodiment, the compounds provided herein may be administered by continuous infusion into the central nervous system. Non-limiting examples of methods that may be used to deliver the compounds provided herein into the central nervous system by continuous infusion may include pumps, wafers, gels, foams and fibrin clots. In a preferred embodiment, the compounds provided herein may be delivered into the central nervous system by continuous infusion using an osmotic pump. An osmotic mini pump contains a high-osmolality chamber that surrounds a flexible, yet impermeable, reservoir filled with the targeted delivery composition-containing vehicle. Subsequent to the subcutaneous implantation of this minipump, extracellular fluid enters through an outer semi-permeable membrane into the high-osmolality chamber, thereby compressing the reservoir to release the targeted delivery composition at a controlled, pre-determined rate. The targeted delivery composition, released from the pump, may be directed via a catheter to a stereotaxically placed cannula for infusion into the cerebroventricular space. In certain embodiments, the compounds provided herein may be delivered into the central nervous system by continuous infusion using a pump as described in the Examples.

In another preferred embodiment, the compounds provided herein may be delivered into the central nervous system by intrathecal administration. A catheter may be placed in the intrathecal lumbar space of the animal. The proximal end of the catheter may be attached to a dosing pedestal that may extend through the

skin. In further embodiments, the compounds provided herein may be administered as a bolus injection. In other embodiments, the compounds provided herein may be administered as a continuous infusion.

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 Tau nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commerical vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, SH-SY5Y and A172.

In vitro testing of antisense oligonucleotides

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

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

One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN (Invitrogen, Carlsbad, CA). Antisense oligonucleotides may be mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final

concentration of antisense oligonucleotide and a LIPOFECTIN concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

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

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

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

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

RNA Isolation

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

Analysis of inhibition of target levels or expression

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

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

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

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

Probes and primers are designed to hybridize to a Tau 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).

Quantitative Real-Time PCR Analysis of Target DNA Levels

Quantitation of target DNA 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.

Gene (or DNA) 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 DNA 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 DNA is quantified using RIBOGREEN RNA quantification reagent (Invitrogen, Inc. Eugene, OR). Methods of DNA quantification by RIBOGREEN are Taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN fluorescence.

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

Analysis of Protein Levels

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

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of Tau and produce phenotypic changes. Testing may be performed in non-transgenic animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, subcutaneous, intrathecal, and intracerebroventricular. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from brain tissue and changes in Tau nucleic acid expression are measured. Changes in Tau DNA levels are also measured. Changes in Tau protein levels are also measured. Changes in Tau splicing are also measured.

Tau splicing

Certain embodiments provided herein relate to differential splicing in tau. Accordingly, several embodiments provide methods of treating a tau associated disease by lowering tau or altering the splicing of a nucleic acid encoding tau. Tau is a protein found in multiple tissues, but is particularly abundant in axons of neurons. The primary function of tau is to bind to and stabilize microtubules, which are important structural components of the cytoskeleton involved in mitosis, cytokinesis and vesicular transport. In humans, there are six isoforms of tau that are generated by alternative splicing of exons 2, 3, and 10. Splicing of exons 2 and 3 at the N-terminus of the protein leads to inclusion of zero, one or two 29 amino acid, acidic domains and is termed 0N, 1 N, or 2N tau respectively. Inclusion of exon 10 at the C-terminus leads to inclusion of the microtubule binding domain encoded by exon 10. Since there are 3 mictrotubule binding domains elsewhere in tau, this tau isoform (with exon 10 included) is termed 4R tau, where R refers to the number of repeats of microtubule binding domains. Tau without exon 10 is termed 3R tau. In healthy subjects, the ratio of 3R:4R tau is developmentally regulated, with fetal tissues expressing exclusively 3R tau and adult human tissues

expressing approximately equal levels of 3R/4R tau. Deviations from the normal ratio of 3R:4R tau are characteristic of neurodegenerative syndromes such as FTD tauopathies. In essence, the method decreases the 4R:3R tau ratio in the central nervous system of the subject.

The 4R:3R tau ratio in the central nervous system of the subject may be normal, low or high. As used herein, a "normal 4R:3R tau ratio" in the central nervous system signifies a 4R:3R tau ratio in the central nervous system that is substantially the same as the 4R:3R tau ratio in the central nervous system of a subject from the same species and of approximately the same age not suffering from a neurodegenerative disease. In some embodiments, the method decreases the normal 4R:3R tau ratio in the central nervous system of a subject. In other embodiments, the method decreases a low 4R:3R tau ratio in the central nervous system of a subject.

In certain embodiments, the method decreases a high 4R:3R tau ratio in the central nervous system of a subject. In certain embodiments, the method decreases a high 4R:3R tau ratio caused by a defect in splicing of the nucleic acid encoding tau in the subject. Defects in splicing of the nucleic acid encoding tau in the subject may be caused, for instance, by genetic mutations altering the splicing of the nucleic acid encoding tau and leading to a high 4R:3R tau ratio. A mutation may be either a substitution mutation or a deletion mutation which creates a new, aberrant, splice element. Non-limiting examples of genetic mutations that may alter the splicing of the nucleic acid encoding tau and lead to a high 4R:3R tau ratio may include N279K, P301S, *Ll280,* L284L, N296H, N296N, Ll296N, P301 S, G303V, E10+11, E1 0+12, E1 0+13, E+1 0+14 and E10+16, and E10+19. Certain embodiments relate to a method of decreasing the 4R:3R tau ratio in the central nervous system of a subject by lowering expression of tau or altering the splicing of a nucleic acid encoding tau administering an antisense compound to the subject.

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, Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), Epilepsy, and Dravet's Syndrome. In certain embodiments, the individual has been identified as having a Tau associated disease. In certain embodiments, provided herein are methods for prophylactically reducing Tau expression in an individual. In certain embodiments, provided herein are methods for prophylactically modulating Tau splicing 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 Tau nucleic acid.

In certain embodiments, administration of a therapeutically effective amount of an antisense compound targeted to a Tau nucleic acid is accompanied by monitoring of Tau levels and Tau isoform 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 Tau nucleic acid results in reduction of Tau 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 Tau nucleic acid results in reduction of the 4R isoform of Tau 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 Tau nucleic acid results in reduced memory loss, reduced anxiety, improved motor function in an animal, and/or reduced incidence or severity of seizures. In certain embodiments, administration of a Tau antisense results in reduced memory loss, reduced anxiety, improved motor function; and/or reduced incidence or severity of seizures by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to Tau are used for the preparation of a medicament for treating a patient suffering or susceptible to a neurodegenerative disease including Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), Epilepsy, and Dravet's Syndrome.

Certain Splicing Compounds

In certain embodiments, splicing compounds are useful for treating neurodegenerative syndromes. In certain embodiments, such splicing compounds promote the exclusion of exon 10, resulting in shifting tau isoform from 4R Tau (which is associated with neurodegenerative syndrome) to 3R Tau. In certain embodiments, such splicing compounds are antisense oligonucleotides wherein each nucleoside comprises a high affinity modification. In certain embodiments, the splicing compound is complementary to a human Tau genetic sequence. In certain embodiments, the splicing compound is complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_010783.14 truncated from nucleotides 2624000 to 2761000).

In certain embodiments, splicing compounds promote the exclusion of exon 1, 5, 7, 9, or 11, resulting in introduction of a pre-mature termination codon. In such embodiments, the resulting mRNA may be targeted for degradation by nonsense mediated decay.

In certain embodiments, splicing compounds promote the exclusion or inclusion of exon -1, 1, 2, 4, 5, 7, 9, 11, 12, or 13, resulting in an aberrant mRNA that is recognized and degraded by the cellular machinery.

EXAMPLES

Non-limiting disclosure and incorporation by reference

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

Example 1: Design of Antisense Compounds Targeted to Tau

A series of modified oligonucleotides were designed to target the Tau transcript, see Table 1 and Table 2 below. These modified oligonucleotides are 18 nucleosides in length, and each nucleoside of the modified oligonucleotide has a 2'-MOE modification. Each internucleoside linkage throughout the modified oligonucleotide are phosphorothioate internucleoside linkages (P=S). All cytosine residues throughout the modified oligonucleotides are 5-methylcytosines. Each modified oligonucleotide listed in Table 1 and Table 2 below is targeted to GENBANK Accession No NT_165773.2 truncated from nucleotides 15633000 to 15736000 (SEQ ID NO: 12). The locations of the target regions on the target Tau transcript are defined in Table A below. Some modified oligonucleotides listed in Tables 1 and 2 have a target region that is completely within an intron. The target regions of such modified oligonucleotides are listed as being near the 3'-end or the 5'-end of the appropriate intron. For example, a modified oligonucleotide with a target region of "5' Intron 1" is targeted to the 5'-end of Intron 1.

Table A: Target Regions on SEQ ID NO: 12

Target Region	SEQ ID: 12 Start Site	SEQ ID: 12 Stop Site
Exon -1	826	997
Intron -1	998	51757
Exon 1	51758	51870
Intron 1	51871	56512
Exon 2	56513	56599
Intron 2	56600	59297
Exon 3	59298	59384
Intron 3	59385	64248

Exon 4	64249	64314
Intron 4	64315	71750
Exon 5	71751	71800
Intron 5-7	71801	76015
Exon 7	76016	76148
Intron 7-9	76149	79658
Exon 9	79659	79924
Intron 9	79925	87546
Exon 10	87547	87639
Intron 10	87640	90740
Exon 11	90741	90822
Intron 11	90823	91817
Exon 12	91818	91930
Intron 12	91931	97361
Exon 13	97362	101486

Table 1: Uniform 2'-MOE modified oligonucleotides targeted to tau

Isis No.	ASO seq.	Target Region	SEQ ID: 12 Start Site	SEQ ID: 12 Stop Site	SEQ ID NO
580255	TTGTGGCGGCGGCGGCAG	Exon -1	928	945	17
580256	GGTGGTTGTGGCGGCGGC	Exon -1	933	950	18
580257	GAGAAGGTGGTTGTGGCG	Exon -1	938	955	19
580258	CGGAGGAGAAGGTGGTTG	Exon -1	943	960	20
580259	GACAGCGGAGGAGAAGGT	Exon -1	948	965	21
580260	AAGAGGACAGCGGAGGAG	Exon -1	953	970	22
580261	GACAGAAGAGGACAGCGG	Exon -1	958	975	23
580262	GCGAGGACAGAAGAGGAC	Exon -1	963	980	24
580263	AGAAGGCGAGGACAGAAG	Exon -1	968	985	25
580264	TCGACAGAAGGCGAGGAC	Exon -1	973	990	26
580265	GATAATCGACAGAAGGCG	Exon -1	978	995	27
580266	TACCTGATAATCGACAGA	Exon -1 / Intron -1	983	1000	28
580267	GCGCTTACCTGATAATCG	Exon -1 / Intron -1	988	1005	29
580268	CTGCGGCGCTTACCTGAT	Exon -1 / Intron -1	993	1010	30
580269	CAGAGCTGCGGCGCTTAC	5' Intron -1	998	1015	31
580270	GATTTCAGAGCTGCGGCG	5' Intron -1	1003	1020	32
580271	GACTGGATTTCAGAGCTG	5' Intron -1	1008	1025	33
580272	AATTGGTTACAGGTGGGA	3' Intron -1	51718	51735	34
580273	AATGGAATTGGTTACAGG	3' Intron -1	51723	51740	35

580274	AAGACAATGGAATTGGTT	3' Intron -1	51728	51745	36
580275	AAAAAAAGACAATGGAAT	3' Intron -1	51733	51750	37
580276	GGAGGAAAAAAAGACAAT	3' Intron -1	51738	51755	38
580277	AGCCTGGAGGAAAAAAAG	Intron -1 / Exon 1	51743	51760	39
580278	TTCAAAGCCTGGAGGAAA	Intron -1 / Exon 1	51748	51765	40
580279	ACTGGTTCAAAGCCTGGA	Intron -1 / Exon 1	51753	51770	41
580280	GCCATACTGGTTCAAAGC	Exon 1	51758	51775	42
580281	GGTCAGCCATACTGGTTC	Exon 1	51763	51780	43
580282	GCGAGGGTCAGCCATACT	Exon 1	51768	51785	44
580283	TCCTGGCGAGGGTCAGCC	Exon 1	51773	51790	45
580284	CAAACTCCTGGCGAGGGT	Exon 1	51778	51795	46
580285	TGTGTCAAACTCCTGGCG	Exon 1	51783	51800	47
580286	TCCATTGTGTCAAACTCC	Exon 1	51788	51805	48
580287	GGTCTTCCATTGTGTCAA	Exon 1	51793	51810	49
580288	AGCATGGTCTTCCATTGT	Exon 1	51798	51815	50
580289	TCTCCAGCATGGTCTTCC	Exon 1	51803	51820	51
580290	TGTAATCTCCAGCATGGT	Exon 1	51808	51825	52
580291	CAGAGTGTAATCTCCAGC	Exon 1	51813	51830	53
580292	TGGAGCAGAGTGTAATCT	Exon 1	51818	51835	54
580293	GGTCTTGGAGCAGAGTGT	Exon 1	51823	51840	55
580294	TTCTTGGTCTTGGAGCAG	Exon 1	51828	51845	56
580295	TCTCCTTCTTGGTCTTGG	Exon 1	51833	51850	57
580296	CCATGTCTCCTTCTTGGT	Exon 1	51838	51855	58
580297	ATGGTCCATGTCTCCTTC	Exon 1	51843	51860	59
580298	AAGCCATGGTCCATGTCT	Exon 1	51848	51865	60
580299	CTTTTAAGCCATGGTCCA	Exon 1	51853	51870	61
580300	CTGACCTTTTAAGCCATG	Exon 1 / Intron 1	51858	51875	62
580301	CCCCACTGACCTTTTAAG	Exon 1 / Intron 1	51863	51880	63
580302	GTCCACCCCACTGACCTT	Exon 1 / Intron 1	51868	51885	64
580303	TGTATGTCCACCCCACTG	5' Intron 1	51873	51890	65
580304	AGTTGTGTATGTCCACCC	5' Intron 1	51878	51895	66
580305	TGGCCAGTTGTGTATGTC	5' Intron 1	51883	51900	67
580306	GTGACTGGCCAGTTGTGT	5' Intron 1	51888	51905	68
580307	CCACTGTGACTGGCCAGT	5' Intron 1	51893	51910	69
580308	TCAGACCTCAAGAGGAAC	3' Intron 1	56473	56490	70
580309	AGCCCTCAGACCTCAAGA	3' Intron 1	56478	56495	71
580310	CAGTGAGCCCTCAGACCT	3' Intron 1	56483	56500	72
580311	ATATACAGTGAGCCCTCA	3' Intron 1	56488	56505	73
580312	GGAACATATACAGTGAGC	3' Intron 1	56493	56510	74
580313	ACTCTGGAACATATACAG	Intron 1 / Exon 2	56498	56515	75

580314	GGGAGACTCTGGAACATA	Intron 1 / Exon 2	56503	56520	76
580315	CTCCGCTCCATCATCGGC	Exon 2	56533	56550	77
580316	GGTTCCTCCGCTCCATCA	Exon 2	56538	56555	78
580317	ACCCTGGTTCCTCCGCTC	Exon 2	56543	56560	79
580318	CTCCGACCCTGGTTCCTC	Exon 2	56548	56565	80
580319	GAGGTCTCCGACCCTGGT	Exon 2	56553	56570	81
580320	CATCGGAGGTCTCCGACC	Exon 2	56558	56575	82
580321	CTTAGCATCGGAGGTCTC	Exon 2	56563	56580	83
580322	GTGCTCTTAGCATCGGAG	Exon 2	56568	56585	84
580323	TTGGAGTGCTCTTAGCAT	Exon 2	56573	56590	85
580324	AGCAGTTGGAGTGCTCTT	Exon 2	56578	56595	86
580325	CCTTCAGCAGTTGGAGTG	Exon 2 / Intron 2	56583	56600	87
580326	CCCCACCTTCAGCAGTTG	Exon 2 / Intron 2	56588	56605	88
580327	GGAGGCCCCACCTTCAGC	Exon 2 / Intron 2	56593	56610	89
580328	AGTAGGGAGGCCCCACCT	Exon 2 / Intron 2	56598	56615	90
580329	GTGGGAGTAGGGAGGCCC	5' Intron 2	56603	56620	91
580330	GAGTGGTGGGAGTAGGGA	5' Intron 2	56608	56625	92
580331	GAATGGAGTGGTGGGAGT	5' Intron 2	56613	56630	93
580332	TGAATGAATGGAGTGGTG	5' Intron 2	56618	56635	94
580333	TAAGCTGAATGAATGGAG	5' Intron 2	56623	56640	95
580334	GAGGACAGAAGGGAGACT	3' Intron 3	64209	64226	96
580335	AGGGAGAGGACAGAAGGG	3' Intron 3	64214	64231	97
580336	AGCAAAGGGAGAGGACAG	3' Intron 3	64219	64236	98
580337	GAGGCAGCAAAGGGAGAG	3' Intron 3	64224	64241	99
580338	GGTCCGAGGCAGCAAAGG	3' Intron 3	64229	64246	100
580339	CGGCTGGTCCGAGGCAGC	Intron 3 / Exon 4	64234	64251	101
580340	TTCTTCGGCTGGTCCGAG	Intron 3 / Exon 4	64239	64256	102
580341	CCTGCTTCTTCGGCTGGT	Intron 3 / Exon 4	64244	64261	103
580342	CGATGCCTGCTTCTTCGG	Exon 4	64249	64266	104
580343	GTCTCCGATGCCTGCTTC	Exon 4	64254	64271	105
580344	TGGTCCTCCTGGTTCGGG	Exon 4	64274	64291	106
580345	CGGCTTGGTCCTCCTGGT	Exon 4	64279	64296	107
580346	CCCAGCGGCTTGGTCCTC	Exon 4	64284	64301	108
580347	ACATGCCCAGCGGCTTGG	Exon 4	64289	64306	109
580348	GAGTCACATGCCCAGCGG	Exon 4	64294	64311	110
580349	ACCTTGAGTCACATGCCC	Exon 4 / Intron 4	64299	64316	111
580350	CACTGACCTTGAGTCACA	Exon 4 / Intron 4	64304	64321	112
580351	TGGGCCACTGACCTTGAG	Exon 4 / Intron 4	64309	64326	113
580352	CGATTTGGGCCACTGACC	Exon 4 / Intron 4	64314	64331	114
580353	AAAGTCGATTTGGGCCAC	5' Intron 4	64319	64336	115

580354	GTCCCAAAGTCGATTTGG	5' Intron 4	64324	64341	116
580355	TCTGAGTCCCAAAGTCGA	5' Intron 4	64329	64346	117
580356	CCCAATCTGAGTCCCAAA	5' Intron 4	64334	64351	118
580357	CTCCTCCCAATCTGAGTC	5' Intron 4	64339	64356	119
580358	GTGCACGTGATGCCCCGT	3' Intron 4	71711	71728	120
580359	AGAGAGTGCACGTGATGC	3' Intron 4	71716	71733	121
580360	AAAATAGAGAGTGCACGT	3' Intron 4	71721	71738	122
580361	AATATAAAATAGAGAGTG	3' Intron 4	71726	71743	123
580362	GGTAAAATATAAAATAGA	3' Intron 4	71731	71748	124
580363	GAGCTGGTAAAATATAAA	Intron 4 / Exon 5	71736	71753	125
580364	CACACGAGCTGGTAAAAT	Intron 4 / Exon 5	71741	71758	126
580365	CTGGCCACACGAGCTGGT	Intron 4 / Exon 5	71746	71763	127
580366	CTTTGCTGGCCACACGAG	Exon 5	71751	71768	128
580367	CCTGTCTTTGCTGGCCAC	Exon 5	71756	71773	129
580368	CCTGTCCTGTCTTTGCTG	Exon 5	71761	71778	130
580369	CATTTCCTGTCCTGTCTT	Exon 5	71766	71783	131
580370	CTCGTCATTTCCTGTCCT	Exon 5	71771	71788	132
580371	TTCTTCTCGTCATTTCCT	Exon 5	71776	71793	133
580372	TGGCTTTCTTCTCGTCAT	Exon 5	71781	71798	134
580373	TACCTTGGCTTTCTTCTC	Exon 5 / Intron 5-7	71786	71803	135
580374	TAGCTTACCTTGGCTTTC	Exon 5 / Intron 5-7	71791	71808	136
580375	GTCAGTAGCTTACCTTGG	Exon 5 / Intron 5-7	71796	71813	137
580376	GGCGGGTCAGTAGCTTAC	5' Intron 5-7	71801	71818	138
580377	GGACCGGCGGGTCAGTAG	5' Intron 5-7	71806	71823	139
580378	TTCTAGGACCGGCGGGTC	5' Intron 5-7	71811	71828	140
580379	GAGCCTTCTAGGACCGGC	5' Intron 5-7	71816	71833	141
580380	AAGCAGAGCCTTCTAGGA	5' Intron 5-7	71821	71838	142
580381	GCAGGAAGCAGAGCCTTC	5' Intron 5-7	71826	71843	143
580382	AGGTTTTCACCACTGGGA	3' Intron 5-7	75976	75993	144
580383	GTGGAAGGTTTTCACCAC	3' Intron 5-7	75981	75998	145
580384	TCAGGGTGGAAGGTTTTC	3' Intron 5-7	75986	76003	146
580385	CCGAATCAGGGTGGAAGG	3' Intron 5-7	75991	76008	147
580386	GTAAACCGAATCAGGGTG	3' Intron 5-7	75996	76013	148
580387	GCCCTGTAAACCGAATCA	Intron 5-7 / Exon 7	76001	76018	149
580388	TCAGCGCCCTGTAAACCG	Intron 5-7 / Exon 7	76006	76023	150
580389	TGCCATCAGCGCCCTGTA	Intron 5-7 / Exon 7	76011	76028	151
580390	GGTTTTGCCATCAGCGCC	Exon 7	76016	76033	152
580391	GCCCCGGTTTTGCCATCA	Exon 7	76021	76038	153
580392	TCTTCGCCCCGGTTTTGC	Exon 7	76026	76043	154
580393	GGCGATCTTCGCCCCGGT	Exon 7	76031	76048	155

580394	GGTGTGGCGATCTTCGCC	Exon 7	76036	76053	156
580395	CCCGAGGTGTGGCGATCT	Exon 7	76041	76058	157
580396	TGCTCCCCGAGGTGTGGC	Exon 7	76046	76063	158
580397	GAGGCTGCTCCCCGAGGT	Exon 7	76051	76068	159
580398	CCGGAGAGGCTGCTCCCC	Exon 7	76056	76073	160
580399	CTGGGCCGGAGAGGCTGC	Exon 7	76061	76078	161
580400	CCCTTCTGGGCCGGAGAG	Exon 7	76066	76083	162
580401	ACGTGCCCTTCTGGGCCG	Exon 7	76071	76088	163
580402	GTTGGACGTGCCCTTCTG	Exon 7	76076	76093	164
580403	GTGGCGTTGGACGTGCCC	Exon 7	76081	76098	165
580404	TCCTGGTGGCGTTGGACG	Exon 7	76086	76103	166
580405	CGGGATCCTGGTGGCGTT	Exon 7	76091	76108	167
580406	TTGGCCGGGATCCTGGTG	Exon 7	76096	76113	168
580407	TGGTCTTGGCCGGGATCC	Exon 7	76101	76118	169
580408	GGGCGTGGTCTTGGCCGG	Exon 7	76106	76123	170
580409	GGGCTGGGCGTGGTCTTG	Exon 7	76111	76128	171
580410	TCTTAGGGCTGGGCGTGG	Exon 7	76116	76133	172
580411	AGGAGTCTTAGGGCTGGG	Exon 7	76121	76138	173
580412	CCTGGAGGAGTCTTAGGG	Exon 7	76126	76143	174
580413	CTGACCCTGGAGGAGTCT	Exon 7	76131	76148	175
580414	CTCACCTGACCCTGGAGG	Exon 7 / Intron 7-9	76136	76153	176
580415	GTAGTCTCACCTGACCCT	Exon 7 / Intron 7-9	76141	76158	177
580416	AGAGAGTAGTCTCACCTG	Exon 7 / Intron 7-9	76146	76163	178
580417	ACTCCAGAGAGTAGTCTC	5' Intron 7-9	76151	76168	179
580418	TTAAAACTCCAGAGAGTA	5' Intron 7-9	76156	76173	180
580419	CTGGATTAAAACTCCAGA	5' Intron 7-9	76161	76178	181
580420	AGCTTCTGGATTAAAACT	5' Intron 7-9	76166	76183	182
580421	CTGGAAGCTTCTGGATTA	5' Intron 7-9	76171	76188	183
580422	GGGACTGAGGCCACGTGG	3' Intron 7-9	79619	79636	184
580423	AGGAAGGGACTGAGGCCA	3' Intron 7-9	79624	79641	185
580424	GAGAGAGGAAGGGACTGA	3' Intron 7-9	79629	79646	186
580425	GTCGGGAGAGAGGAAGGG	3' Intron 7-9	79634	79651	187
580426	GGAAAGTCGGGAGAGAGG	3' Intron 7-9	79639	79656	188
580427	CACCTGGAAAGTCGGGAG	Intron 7-9 / Exon 9	79644	79661	189
580428	TGGTTCACCTGGAAAGTC	Intron 7-9 / Exon 9	79649	79666	190
580429	TTTGGTGGTTCACCTGGA	Intron 7-9 / Exon 9	79654	79671	191
580430	CGGATTTTGGTGGTTCAC	Exon 9	79659	79676	192
580431	TTCTCCGGATTTTGGTGG	Exon 9	79664	79681	193
580432	CTTCGTTCTCCGGATTTT	Exon 9	79669	79686	194
580433	AGCCGCTTCGTTCTCCGG	Exon 9	79674	79691	195

580434	GCTGTAGCCGCTTCGTTC	Exon 9	79679	79696	196
580435	GGGCTGCTGTAGCCGCTT	Exon 9	79684	79701	197
580436	CAGGCGTTCCGGGAGAGC	Exon 9	79704	79721	198
580437	ACTGCCAGGCGTTCCGGG	Exon 9	79709	79726	199
580438	GAGCGACTGCCAGGCGTT	Exon 9	79714	79731	200
580439	TGCGCGAGCGACTGCCAG	Exon 9	79719	79736	201
580440	CGGTGTTGGTAGGGATGG	Exon 9	79739	79756	202
580441	GTGGGCGGTGTTGGTAGG	Exon 9	79744	79761	203
580442	CCCGGGTGGGCGGTGTTG	Exon 9	79749	79766	204
580443	GGGCTCCCGGGTGGGCGG	Exon 9	79754	79771	205
580444	TTCTTGGGCTCCCGGGTG	Exon 9	79759	79776	206
580445	CCACCTTCTTGGGCTCCC	Exon 9	79764	79781	207
580446	CACTGCCACCTTCTTGGG	Exon 9	79769	79786	208
580447	CGGACCACTGCCACCTTC	Exon 9	79774	79791	209
580448	GAGTGCGGACCACTGCCA	Exon 9	79779	79796	210
580449	ATGGTGACTTAGGGGGAG	Exon 9	79794	79811	211
580450	AGCTGATGGTGACTTAGG	Exon 9	79799	79816	212
580451	TTACTAGCTGATGGTGAC	Exon 9	79804	79821	213
580452	GGCTCTTACTAGCTGATG	Exon 9	79809	79826	214
580453	CAGGCGGCTCTTACTAGC	Exon 9	79814	79831	215
580454	GTCTGCAGGCGGCTCTTA	Exon 9	79819	79836	216
580455	GGGCAGTCTGCAGGCGGC	Exon 9	79824	79841	217
580456	TAGGTCTGGCATGGGCAC	Exon 9	79844	79861	218
580457	TTCTTTAGGTCTGGCATG	Exon 9	79849	79866	219
580458	TGACATTCTTTAGGTCTG	Exon 9	79854	79871	220
580459	CGACCTGACATTCTTTAG	Exon 9	79859	79876	221
580460	ATCTTCGACCTGACATTC	Exon 9	79864	79881	222
580461	AGCCAATCTTCGACCTGA	Exon 9	79869	79886	223
580462	AGTAGAGCCAATCTTCGA	Exon 9	79874	79891	224
580463	TTCTCAGTAGAGCCAATC	Exon 9	79879	79896	225
580464	TCAGGTTCTCAGTAGAGC	Exon 9	79884	79901	226
580465	GTGCTTCAGGTTCTCAGT	Exon 9	79889	79906	227
580466	GGCTGGTGCTTCAGGTTC	Exon 9	79894	79911	228
580467	CTCCTGGCTGGTGCTTCA	Exon 9	79899	79916	229
580468	GCCACCTCCTGGCTGGTG	Exon 9	79904	79921	230
580469	ACCTTGCCACCTCCTGGC	Exon 9 / Intron 9	79909	79926	231
580470	CCCTTACCTTGCCACCTC	Exon 9 / Intron 9	79914	79931	232
580471	CCACACCCTTACCTTGCC	Exon 9 / Intron 9	79919	79936	233
580472	ACCAGCCACACCCTTACC	Exon 9 / Intron 9	79924	79941	234
580473	AGAAGACCAGCCACACCC	5' Intron 9	79929	79946	235

580474	TTCCCAGAAGACCAGCCA	5' Intron 9	79934	79951	236
580475	CTGGCTTCCCAGAAGACC	5' Intron 9	79939	79956	237
580476	GGAGTCTGGCTTCCCAGA	5' Intron 9	79944	79961	238
580477	AGGTGGGAGTCTGGCTTC	5' Intron 9	79949	79966	239
580478	GAGAGAGAGAATAAAGGG	3' Intron 10	90701	90718	240
580479	TGGGCGAGAGAGAGAATA	3' Intron 10	90706	90723	241
580480	GAGGATGGGCGAGAGAGA	3' Intron 10	90711	90728	242
580481	TCAAAGAGGATGGGCGAG	3' Intron 10	90716	90733	243
580482	GCAGGTCAAAGAGGATGG	3' Intron 10	90721	90738	244
580483	CACCTGCAGGTCAAAGAG	Intron 10 / Exon 11	90726	90743	245
580484	ATTTGCACCTGCAGGTCA	Intron 10 / Exon 11	90731	90748	246
580485	AGACTATTTGCACCTGCA	Intron 10 / Exon 11	90736	90753	247
580486	CTTGTAGACTATTTGCAC	Exon 11	90741	90758	248
580487	ACCGGCTTGTAGACTATT	Exon 11	90746	90763	249
580488	GGTCCACCGGCTTGTAGA	Exon 11	90751	90768	250
580489	GCTCAGGTCCACCGGCTT	Exon 11	90756	90773	251
580490	ACTTTGCTCAGGTCCACC	Exon 11	90761	90778	252
580491	AGGTCACTTTGCTCAGGT	Exon 11	90766	90783	253
580492	CTTGGAGGTCACTTTGCT	Exon 11	90771	90788	254
580493	CCACACTTGGAGGTCACT	Exon 11	90776	90793	255
580494	ACGAGCCACACTTGGAGG	Exon 11	90781	90798	256
580495	CCCTAACGAGCCACACTT	Exon 11	90786	90803	257
580496	ATGTTCCCTAACGAGCCA	Exon 11	90791	90808	258
580497	GATGGATGTTCCCTAACG	Exon 11	90796	90813	259
580498	CTTGTGATGGATGTTCCC	Exon 11	90801	90818	260
580499	CCTGGCTTGTGATGGATG	Exon 11 / Intron 11	90806	90823	261
580500	TGCTACCTGGCTTGTGAT	Exon 11 / Intron 11	90811	90828	262
580501	TCAAGTGCTACCTGGCTT	Exon 11 / Intron 11	90816	90833	263
580502	CTTCCTCAAGTGCTACCT	Exon 11 / Intron 11	90821	90838	264
580503	CCTGTCTTCCTCAAGTGC	5' Intron 11	90826	90843	265
580504	CAAAGCCTGTCTTCCTCA	5' Intron 11	90831	90848	266
580505	TGTCCCAAAGCCTGTCTT	5' Intron 11	90836	90853	267
580506	ACTCCTGTCCCAAAGCCT	5' Intron 11	90841	90858	268
580507	CCAGCACTCCTGTCCCAA	5' Intron 11	90846	90863	269
580508	CTCTTGCCCTAGTCTGTG	3' Intron 11	91778	91795	270
580509	TGAGCCTCTTGCCCTAGT	3' Intron 11	91783	91800	271
580510	CCACATGAGCCTCTTGCC	3' Intron 11	91788	91805	272
580511	ACAACCCACATGAGCCTC	3' Intron 11	91793	91810	273
580512	GGAACACAACCCACATGA	3' Intron 11	91798	91815	274
580513	CTCCTGGAACACAACCCA	Intron 11 / Exon 12	91803	91820	275

580514	GCCACCTCCTGGAACACA	Intron 11 / Exon 12	91808	91825	276
580515	ACCTGGCCACCTCCTGGA	Intron 11 / Exon 12	91813	91830	277
580516	CTTCCACCTGGCCACCTC	Exon 12	91818	91835	278
580517	TTTTACTTCCACCTGGCC	Exon 12	91823	91840	279
580518	TCTGATTTTACTTCCACC	Exon 12	91828	91845	280
580519	GCTTCTCTGATTTTACTT	Exon 12	91833	91850	281
580520	GTCCAGCTTCTCTGATTT	Exon 12	91838	91855	282
580521	TTGAAGTCCAGCTTCTCT	Exon 12	91843	91860	283
580522	TGTCCTTGAAGTCCAGCT	Exon 12	91848	91865	284
580523	GACTCTGTCCTTGAAGTC	Exon 12	91853	91870	285
580524	GACTGGACTCTGTCCTTG	Exon 12	91858	91875	286
580525	TCTTCGACTGGACTCTGT	Exon 12	91863	91880	287
580526	GCCAATCTTCGACTGGAC	Exon 12	91868	91885	288
580527	AAGGAGCCAATCTTCGAC	Exon 12	91873	91890	289
580528	TATCCAAGGAGCCAATCT	Exon 12	91878	91895	290
580529	GATATTATCCAAGGAGCC	Exon 12	91883	91900	291
580530	TGGGTGATATTATCCAAG	Exon 12	91888	91905	292
580531	GGACGTGGGTGATATTAT	Exon 12	91893	91910	293
580532	TCCAGGGACGTGGGTGAT	Exon 12	91898	91915	294
580533	CCTCCTCCAGGGACGTGG	Exon 12	91903	91920	295
580534	TATTCCCTCCTCCAGGGA	Exon 12	91908	91925	296
580535	CTTCTTATTCCCTCCTCC	Exon 12	91913	91930	297
580536	CTTACCTTCTTATTCCCT	Exon 12 / Intron 12	91918	91935	298
580537	ACCCCCTTACCTTCTTAT	Exon 12 / Intron 12	91923	91940	299
580538	ATTTGACCCCCTTACCTT	Exon 12 / Intron 12	91928	91945	300
580539	CTCCCATTTGACCCCCTT	5' Intron 12	91933	91950	301
580540	ATGACCTCCCATTTGACC	5' Intron 12	91938	91955	302
580541	CCCGTATGACCTCCCATT	5' Intron 12	91943	91960	303
580542	TTATCCCCGTATGACCTC	5' Intron 12	91948	91965	304
580543	CCCTCTTATCCCCGTATG	5' Intron 12	91953	91970	305
580544	TTAATGATAAGGTTCTAT	3' Intron 12	97322	97339	306
580545	TGAGATTAATGATAAGGT	3' Intron 12	97327	97344	307
580546	GAGAGTGAGATTAATGAT	3' Intron 12	97332	97349	308
580547	ATGTAGAGAGTGAGATTA	3' Intron 12	97337	97354	309
580548	GCAAGATGTAGAGAGTGA	3' Intron 12	97342	97359	310
580549	AATCTGCAAGATGTAGAG	Intron 12 / Exon 13	97347	97364	311
580550	GTTTCAATCTGCAAGATG	Intron 12 / Exon 13	97352	97369	312
580551	TGTGGGTTTCAATCTGCA	Intron 12 / Exon 13	97357	97374	313
580552	CAGCTTGTGGGTTTCAAT	Exon 13	97362	97379	314
580553	AAGGTCAGCTTGTGGGTT	Exon 13	97367	97384	315

580554	CCCTGAAGGTCAGCTTGT	Exon 13	97372	97389	316
580555	ATTCTCCCTGAAGGTCAG	Exon 13	97377	97394	317
580556	TTGGCATTCTCCCTGAAG	Exon 13	97382	97399	318
580557	TGGCTTTGGCATTCTCCC	Exon 13	97387	97404	319
580558	TGTCTTGGCTTTGGCATT	Exon 13	97392	97409	320
580559	TGGTCTGTCTTGGCTTTG	Exon 13	97397	97414	321
580560	CTCCATGGTCTGTCTTGG	Exon 13	97402	97419	322
580561	TTCTGCTCCATGGTCTGT	Exon 13	97407	97424	323
580562	ACAATTTCTGCTCCATGG	Exon 13	97412	97429	324

Table 2: Uniform 2'-MOE modified oligonucleotides targeted to tau

Isis No.	ASO seq.	Target Region	SEQ ID: 12 Start Site	SEQ ID: 12 Stop Site	SEQ ID NO
607479	ATCGACAGAAGGCGAGGA	Exon -1	974	991	325
607480	AATCGACAGAAGGCGAGG	Exon -1	975	992	326
607481	TAATCGACAGAAGGCGAG	Exon -1	976	993	327
607482	ATAATCGACAGAAGGCGA	Exon -1	977	994	328
607483	TGATAATCGACAGAAGGC	Exon -1	979	996	329
607484	CTGATAATCGACAGAAGG	Exon -1	980	997	330
607485	CCTGATAATCGACAGAAG	Exon -1/Intron-1	981	998	331
607486	ACCTGATAATCGACAGAA	Exon -1/Intron-1	982	999	332
607487	TTACCTGATAATCGACAG	Exon -1/Intron-1	984	1001	333
607488	CTTACCTGATAATCGACA	Exon -1/Intron-1	985	1002	334
607489	GCTTACCTGATAATCGAC	Exon -1/Intron-1	986	1003	335
607490	CGCTTACCTGATAATCGA	Exon -1/Intron-1	987	1004	336
607491	GGCGCTTACCTGATAATC	Exon -1/Intron-1	989	1006	337
607492	CGGCGCTTACCTGATAAT	Exon -1/Intron-1	990	1007	338
607493	GCGGCGCTTACCTGATAA	Exon -1/Intron-1	991	1008	339
607494	TGCGGCGCTTACCTGATA	Exon -1/Intron-1	992	1009	340
607495	GCTGCGGCGCTTACCTGA	Exon -1/Intron-1	994	1011	341
607496	AGCTGCGGCGCTTACCTG	Exon -1/Intron-1	995	1012	342
607497	GAGCTGCGGCGCTTACCT	Exon -1/Intron-1	996	1013	343
607498	AGAGCTGCGGCGCTTACC	Exon -1/Intron-1	997	1014	344

5

Example 2: Effects of uniform 2'-MOE modified oligonucleotides on Tau expression

Modified oligonucleotides were transfected in B16-F10 cells (7500 cells/well) using 2 mg/ml cytofectin in OPTI-MEM for 4 hours and then lysed 24 hours later for RNA isolation. The Modified oligonucleotides were transfected at 50 nM concentration. RNA isolation was performed using Invitrogen RNAeasy 96 well columns with Dnase I digestion on the column. Total Tau mRNA was analyzed via qRT-PCR reaction with primer probe set RTS3061 (forward sequence 5'- CGGCACCTCAGCAATGTGT-3', SEQ ID NO: 13; reverse sequence 5'- TGTGGCAAGCTGTGGTGAGT-3', SEQ ID NO: 14; probe sequence 5'- TTCCACGGGCAGCATCGACATGX-3', SEQ ID NO: 15). RNA was normalized to Ribogreen. ISIS No. 424880 is a 5-10-5 MOE gapmer having the sequence: ATCACTGATTTTGAAGTCCC (SEQ ID NO: 16); wherein each internucleoside linkage throughout is a phosphorothioate internucleoside linkages (P=S) and all cytosine residues are 5-methylcytosines. Results are presented as percent reduction of RNA expression, relative to untreated control levels and is denoted as "% Reduction" in the tables below. As illustrated in the tables below, modified oligonucleotides targeted to tau, including tau exon/intron junctions, tau introns, tau exons, and tau splice modulation sites reduce tau mRNA. Modified oligonucleotides that modulate splicing resulting in a frameshift and introduction of a premature stop codon likely work, at least in part, to reduce Tau mRNA via nonsense mediated decay. The tolerability of the modified oligonucleotides can be determined using methods, such as those described in WO2010/148249. Like the fully modified 2'-MOE oligonucleotides described in WO2010/148249, the fully modified 2'-MOE oliognucleotides described herein are expected to be tolerable when administered to the CNS.

Table 3: Effect of modified oligonucleotides on Tau expression

Isis #	% Reduction
580335	13
580336	0
580337	17
580338	16
580339	0
580340	0
580341	0
580342	0
580343	8
580344	0
580345	11
580346	5
580347	1
580348	2
580349	0
580350	0

580351	2
580352	11
580353	0
580354	5
580355	0
580356	0
580357	0
580358	0
580359	6
580360	0
580361	22
580362	0
580363	0
580364	0
580365	0
580366	10
580367	17
580368	23
580369	14
580370	17
580371	16
580372	8
580373	8
580374	11
580375	29
580376	9
580377	3
580378	0
580379	0
580380	0
580381	0
580382	12
580383	10
580384	0
580385	32
580386	23
580387	8
580388	13
580389	33
580390	41
580391	10
580392	10
580393	0

580394	19
580395	0
580396	0
580397	14
580398	0
580399	12
580400	0
580401	6
580402	0
580403	0
580404	0
580405	0
580406	0
580407	1
580408	4
580409	8
580410	21
580411	0
580412	7
580413	3
580414	13
424880	84
No Oligo	0

Table 4: Effect of modified oligonucleotides on Tau expression

Isis #	% Reduction
580495	0
580496	6
580497	0
580498	20
580499	36
580500	12
580501	3
580502	0
580503	13
580504	7
580505	0
580506	1
580507	8
580508	27
580509	39

580510	19
580511	0
580512	21
580513	8
580514	9
580515	0
580516	15
580517	23
580518	21
580519	17
580520	16
580521	12
580522	18
580523	0
580524	0
580525	12
580526	1
580527	16
580528	25
580529	8
580530	14
580531	3
580532	0
580533	6
580534	0
580535	32
580536	0
580537	8
580538	0
580539	23
580540	12
580541	12
580542	7
580543	3
580544	3
580545	10
580546	0
580547	16
580548	0
580549	0
580550	19
580551	4
580552	7

580553	5
580554	1
580555	19
580556	0
580557	10
580558	8
580559	0
580560	0
580561	15
580562	11
424880	80
No Oligo	0

Table 5: Effect of modified oligonucleotides on Tau expression

ISIS#	% Reduction
580255	0
580256	0
580257	0
580258	4
580259	4
580260	9
580261	0
580262	14
580263	16
580264	18
580265	46
580266	50
580267	16
580268	56
580269	20
580270	0
580271	0
580272	13
580273	0
580274	16
580275	16
580276	0
580277	2
580278	10

580279	3
580280	8
580281	25
580282	26
580283	13
580284	11
580285	19
580286	12
580287	11
580288	8
580289	26
580290	21
580291	23
580292	28
580293	26
580294	23
580295	0
580296	19
580297	15
580298	0
580299	29
580300	33
580301	16
580302	42
580303	34
580304	19
580305	20
580306	0
580307	0
580308	15
580309	14
580310	12
580311	2
580312	10
580313	8
580314	0
580315	5
580316	3
580317	0
580318	0
580319	0
580320	0
580321	8

580322	7
580323	0
580324	0
580325	3
580326	13
580327	6
580328	8
580329	6
580330	0
580331	0
580332	0
580333	11
580334	3
424880	82
No Oligo	0

Table 6: Effect of modified oligonucleotides on Tau expression

Isis #	% Reduction
580415	21
580416	20
580417	17
580418	8
580419	14
580420	5
580421	4
580422	12
580423	15
580424	7
580425	21
580426	19
580427	5
580428	14
580429	0
580430	38
580431	26
580432	17
580433	3
580434	15
580435	0
580436	0
580437	0

580438	23
580439	7
580440	12
580441	3
580442	0
580443	12
580444	4
580445	8
580446	0
580447	16
580448	11
580449	13
580450	19
580451	15
580452	15
580453	24
580454	8
580455	2
580456	22
580457	19
580458	23
580459	21
580460	20
580461	19
580462	34
580463	36
580464	13
580465	33
580466	15
580467	22
580468	0
580469	20
580470	7
580471	4
580472	16
580473	4
580474	0
580475	11
580476	0
580477	1
580478	7
580479	12
580480	16

580481	9
580482	31
580483	0
580484	0
580485	42
580486	40
580487	41
580488	40
580489	22
580490	34
580491	44
580492	44
580493	46
580494	45
424880	81
No Oligo	0

Table 7: Effect of modified oligonucleotides on Tau expression

Isis #	% Reduction
607479	39
607480	37
607481	34
607482	36
607483	50
607484	65
607485	54
607486	48
607487	47
607488	29
607489	15
607490	1
607491	48
607492	50
607493	66
607494	62
607495	43
607496	38
607497	40
607498	38

424880	85
No Oligo	0

Claims:

1. An antisense compound comprising a modified oligonucleotide consisting of 10-30 linked nucleosides and having a nucleobase sequence complementary to an intron/exon junction or an exon/intron junction or a splice modulation site of a Tau transcript.
2. An antisense compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 17-344.
3. An antisense compound comprising a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 17-344.
4. An antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-344.
5. An antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 9 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-344.
6. An antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-344.
7. An antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 11 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-344.
8. An antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17-344.
9. An antisense compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 17-344.
10. An antisense compound comprising a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 17-344.
11. The antisense compound of any of claims 1-10, wherein the antisense compound is single-stranded.
12. The antisense compound of any of claims 1-10, wherein the antisense compound is double-stranded.
13. The antisense compound of any of claims 1-12, wherein the antisense compound comprises at least one conjugate.
14. The antisense compound of any of claims 1-13, wherein the modified oligonucleotide comprises at least one modified nucleoside.

15. The antisense compound of claim 14, wherein the modified oligonucleotide comprises at least one modified nucleoside comprising a modified sugar.
16. The antisense compound of claim 15, wherein the at least one modified sugar is selected from among a bicyclic sugar, a non-bicyclic 2'-modifed sugar, and a sugar surrogate.
17. The antisense compound of claim 16, wherein at least one modified nucleoside is a 2'-MOE modified nucleoside.
18. The antisense compound of claim 16, wherein at least one modified nucleoside is a morpholino nucleoside.
19. The antisense compound of any of claims 14-18, wherein essentially each nucleoside of the modified oligonucleotide is modified.
20. The antisense compound of any of claims 14-18, wherein each nucleoside of the modified oligonucleotide is modified.
21. The antisense compound of any of claims 14-20, wherein each modified nucleoside has the same modification.
22. The antisense compound of any of claims 14-20, wherein at least two modified nucleoside have different modifications from one another.
23. The antisense compound of any of claims 1-22, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.
24. The antisense compound of claim 23, wherein each internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.
25. The antisense compound of claim 23 or 24, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.
26. The antisense compound of any of claims 1-25, wherein the modified oligonucleotide consists of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 linked nucleosides.
27. The antisense compound of any of claims 1-26, wherein the modified oligonucleotide is at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% complementary to an equal length portion of a human Tau nucleic acid.
28. The antisense compound of any of claims 1-26, wherein the modified oligonucleotide is 100% complementary to an equal length portion of a human Tau nucleic acid.
29. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon -1/intron -1 of the Tau transcript.
30. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron -1/exon 1 of the Tau transcript.
31. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 1/intron 1 of the Tau transcript.
32. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to

the junction of intron 1/exon 2 of the Tau transcript.

33. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 2/intron 2 of the Tau transcript.
34. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 2/exon 3 of the Tau transcript.
35. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 3/intron 3 of the Tau transcript.
36. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 3/exon 4 of the Tau transcript.
37. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 4/intron 4 of the Tau transcript.
38. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 4/exon 5 of the Tau transcript.
39. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 5/intron 5 of the Tau transcript.
40. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 5/exon 6 of the Tau transcript.
41. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 6/intron 6 of the Tau transcript.
42. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 6/exon 7 of the Tau transcript.
43. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 7/intron 7 of the Tau transcript.
44. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 7/exon 8 of the Tau transcript.
45. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 8/intron 8 of the Tau transcript.
46. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 8/exon 9 of the Tau transcript.
47. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 9/intron 9 of the Tau transcript.
48. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 9/exon 10 of the Tau transcript.
49. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 10/intron 10 of the Tau transcript.

50. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 10/exon 11 of the Tau transcript.
51. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 11/intron 11 of the Tau transcript.
52. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 11/exon 12 of the Tau transcript.
53. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 12/intron 12 of the Tau transcript.
54. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of intron 12/exon 13 of the Tau transcript.
55. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to the junction of exon 13/intron 13 of the Tau transcript.
56. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to a splice modulation site within intron -1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 of the Tau transcript.
57. The antisense compound of any of claims 1-28, wherein the modified oligonucleotide is complementary to a splice modulation site within exon -1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 of the Tau transcript.
58. The antisense compound of any of claims 1-57, wherein the Tau transcript is a mouse Tau transcript.
59. The antisense compound of any of claims 1-57, wherein the Tau transcript is a human Tau transcript.
60. The antisense compound of any of claims 1-58, wherein the modified oligonucleotide has a nucleobase sequence comprising at least 8, 10, 12, 14, 16, 18, 20, or 22 contiguous nucleobases of a nucleobase sequence selected from among of any of the oligonucleotides described in the present disclosure.
61. A method comprising contacting a cell with an antisense compound according to any of claims 1-60.
62. The method of claim 61, wherein the cell is in vitro.
63. The method of claim 61, wherein the cell is in an animal.
64. A method for reducing the amount or activity of a Tau transcript in a cell comprising contacting the cell with an antisense compound of any of claims 1-60 and thereby reducing the amount or activity of the Tau transcript in the cell.
65. The method of claim 64, wherein the amount of Tau transcript is reduced.
66. A method of treating a Tau disorder in an animal comprising administering to the animal an antisense compound according to any of claims 1-60.
67. The method of claim 66, wherein the Tau disorder is selected from among: Tauopathy, Alzheimer's Disease, Fronto-temporal Dementia (FTD), FTDP-17, Progressive Supranuclear Palsy (PSP), Chronic Traumatic Encephalopathy (CTE), Corticobasal Ganglionic Degeneration (CBD), Epilepsy, or Dravet's Syndrome.
68. The method of claim 66 or 67, wherein the animal is a mouse.

69. The method of claim 66 or 67, wherein the animal is a human.
70. A method of reducing the amount or activity of a Tau transcript in a cell comprising contacting the cell with an antisense compound, wherein the antisense compound comprises a fully modified oligonucleotide, wherein each nucleoside of the fully modified oligonucleotide comprises the same sugar modification.
71. The method of claim 70, wherein the sugar modification is 2'-MOE.
72. The method of claim 71, wherein the sugar modification is morpholino or 2'-OMethyl.
73. The method of any of claims 70-72, wherein the modified oligonucleotide comprises a phosphorothioate internucleoside linkage.
74. The method of claim 73, wherein each internucleoside linkage of the modified oligonucleotide is a phosphorothioate internucleoside linkage.
75. The method of claim 73, wherein each internucleoside linkage of the modified oligonucleotide is, independently, a phosphate internucleoside linkage or a phosphorothioate internucleoside linkage.
76. The method of any of claims 70-75, wherein the modified oligonucleotide has a sequence selected from among SEQ ID NO: 37, 206, 207, 209, 227, 228, 237, 278, 279, 287, 313, 314, 315, and 324.
77. The method of any of claims 70-76, wherein the cell is in an animal.
78. The method of claim 77, wherein the cell in the central nervous system of the animal.
79. The method of any of claims 77-78, wherein the antisense compound is administered intrathecally to the animal.
80. A method of reducing the amount or activity of a transcript in the central nervous system of an animal comprising intrathecal administration of an antisense compound to the animal, wherein the antisense compound comprises a fully modified oligonucleotide, and wherein the amount or activity of the transcript is reduced via nonsense mediated decay.
81. The method of claim 80, wherein each nucleoside of the fully modified oligonucleotide comprises the same sugar modification.
82. The method of claim 81, wherein the sugar modification is 2'-MOE, 2'-OMethyl, or morpholino.
83. The method of claim 82, wherein the sugar modification is 2'-MOE.
84. The method of any of claims 80-83, wherein the modified oligonucleotide comprises a phosphorothioate internucleoside linkage.
85. The method of claim 84, wherein each internucleoside linkage of the modified oligonucleotide is a phosphorothioate internucleoside linkage.
86. The method of claim 84, wherein each internucleoside linkage of the modified oligonucleotide is, independently, a phosphate internucleoside linkage or a phosphorothioate internucleoside linkage.
87. The method of claim 80, wherein the antisense compound is the antisense compound of any of claims 1-60.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/42740

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/02, A61K 31/7115, C12N 15/113 (2015.01)
CPC - C07H 21/04, C07H 21/02, C12N 2310/11
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): C07H 21/02, A61K 31/7115, C12N 15/113 (2015.01)
CPC: C07H 21/04, C07H 21/02, C12N 2310/11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: C12N2310/34, C12N2310/346, C12N2310/31

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms: antisense compound, siRNA shRNA, RNAi, Tau transcript, Tau Alzheimers, intron/exon junction, exon/intronjunction, splaice modulation site, modified oligonucleotide, single stranded, double stranded, SS, DS, modified sugar, 2'-MOE, 2'-0Methyl, phosphorothioate, CNS, fully modified

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — A	WO 2013/148283 A1 (ISIS PHARMACEUTICALS, INC.) 3 October 2013 (03.10.2013) pg 3, ln 5 -8, pg 25, ln 29-35 - pg 26, ln 1, pg 29, ln 34- pg 30, ln 1, pg 42, ln 6-7, ln 1, pg 46 ln 31-33, pg 50, ln 31-32 - pg 51, ln 1-7	1, (11,12)/1 ---------- 2-3, 9-10, (11-12)/(2-3,9-10)
X — A	US 2011/0263687 A1 (MATTICK et al.) 27 October 2011 (27.10.2011) abstract, para [0005], [0006], [0009], [0062], [0085], [0134], [0136], [0137], Fig. 1, SEQ ID NO: 7124	4-8, (11,12)/(4-8) ---------- 2-3, 9-10, (11-12)/(2-3,9-10)
A	US 2006/216722 A1 (BETSHOLTZ et al.) 28 September 2006 (28.09.2006) para [0184], Tables 3, 6, SEQ ID NOs; 327, 329	2-3, 9-10, (11-12)/(2-3,9-10)
A	WO 2013/148260 A1 (WASHINGTON UNIVERSITY) 3 October 2013 (03.10.2013) abstract, para [0644], [0671]	2-3, 9-10, (11-12)/(2-3,9-10)

☐ 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
"E" earlier application or patent but published on or after the international filing date
"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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 December 2015 (02.12.2015)	29 DEC 2015
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

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/42740

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.: 13-69, 76-79, 87
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:

********** 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.:
1-12, limited to SEQ ID NO: 17

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/42740

Continuation of Box No. III, Observations where unity of invention is lacking:

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. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-12, drawn to an antisense compound comprising a modified oligonucleotide having a nucleobase sequence complementary to a Tau transcript. The antisense compounds will be searched to the extent that the antisense compound encompasses SEQ ID NO: 17. It is believed that claims 1-12, limited to SEQ ID NO: 17, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass the antisense compound comprising modified oligonucleotides having a nucleobase sequence consisting of SEQ ID NO: 17. Additional antisense compound(s) will be searched upon the payment of additional fee(s). Applicants must specify the claims that encompass any additionally elected antisense compound(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 an antisense compound of SEQ ID NO: 18, i.e. claims 1-12.

Group II: Claims 70-75, drawn to a method of reducing the amount or activity of a Tau transcript in a cell by contacting said cell with a fully modified antisense compound.

Group III: Claims 80-86, drawn to a method of reducing the amount or activity of a transcript in the central nervous system of an animal with a fully modified antisense compound.

The inventions listed as Groups I+ through III 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

Group I+ requires an antisense compound having a nucleobase sequence complementary to an intron/exon junction or an exon/intron junction or a splice modulation site of a Tau transcript, which is not required by groups II and III.

Group II requires an antisense compound comprising a fully modified oligonucleotide having the same sugar modification, which is not required by groups I+ and III.

Group III requires a method of reducing the amount or activity of a transcript in the central nervous system via nonsense mediated decay, which is not required by groups I+ and II.

Another special technical feature of the inventions listed as Group I+ is the specific oligonucleotide sequences recited therein. Each invention requires an oligonucleotide sequence not required by any of the other inventions.

Common Technical Features

The common technical feature shared by Groups I+ through III is an antisense compound comprising modified oligonucleotide. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by WO 2013/148283 A1 to Bennett et al. (hereinafter Bennett). Bennett discloses antisense compounds comprising modified oligonucleotides (pg 43, ln 9-13 "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", pg 42, ln 6-7 "In certain embodiments, the single-stranded antisense oligonucleotide comprises at least one modified nucleobase", pg 46 ln 31-33 "In certain embodiments, an antisense compound targeted to a Tau nucleic acid is uniformly modified. ... In certain embodiments, each nucleoside is chemically modified."). As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The common technical feature shared by Groups II and III is reducing the amount or activity of a transcript [e.g. Tau] with a fully modified antisense compound, howvever, Bennett discloses reducing the activity of a Tau transcript wiht antisense compound (abstract "Disclosed herein are methods for reducing expression of Tau mRNA and protein in an animal with Tau antisense compounds", pg 45, ln 19-26 "In certain embodiments, antisense compounds targeted to a Tau nucleic acid have chemically modified subunits ... 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 ... In certain embodiments, the antisense compounds are uniform sugar-modified oligonucleotides.", pg 42, ln 5 "In certain embodiments, each nucleoside is modified).
The common technical feature shared by Group I+ is an antisense compound comprising a modified oligonucleotide consisting of 10-30 linked nucleosides and having a nucleobase sequence complementary to an intron/exon junction or an exon/intron junction or a splice modulation site of a Tau transcript; wherein said oligonucleotide comprises a nucleobase sequece comprising at least, 8, 9, 10, 11, 12 or more, complementary to the target sequence (claims 1-10). However, Bennett discloses said antisense compounds (pg 25, ln 29-35 - pg 26, ln 1 "The antisense oligonucleotide of the invention may disrupt the splicing of the nucleic acid encoding tau ... Each intron is defined by a 5' splice site, a 3' splice site, and a branch point situated there between. An antisense oligonucleotide may block these splice elements when the oligonucleotide either fully or partially overlaps the element, or binds to the pre-mRNA at a position sufficiently close to the element to disrupt the binding and function of the splicing factors which would ordinarily mediate the particular splicing reaction ". pg 50, ln 31-32 - pg 51, ln1-7, "In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment ... Also contemplated are antisense compounds that are complementary to at least a 9, 10, 1 1, 12, 13, 14, ... or more nucleobase portion of a target segment").

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I+ through III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.