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(54) **Title:** LNA OLIGONUCLEOTIDES WITH ALTERNATING FLANKS

(57) **Abstract:** The present invention relates to LNA oligomers having two flanks, wherein one or both flanks comprise alternating LNA and DNA nucleosides.



LNA OLIGONUCLEOTIDES WITH ALTERNATING FLANKS

REFERENCE TO EARLIER FILED APPLICATIONS

[0001] This application is a non-provisional application claiming the benefit of U.S. Provisional Application No. 62/112,058, filed February 4, 2015, U.S. Provisional Application No. 62/156,684, filed May 4, 2015, U.S. Provisional Application No. 62/237,922, filed October 6, 2015, U.S. Provisional Application No. 62/238,941, filed October 8, 2015, and U.S. Provisional Application No. 62/279,614, filed January 15, 2016, all of which are incorporated herein by reference in their entireties.

REFERENCE TO SEQUENCE LISTING SUBMITTED

ELECTRONICALLY VIA EFS-WEB

[0002] The content of the electronically submitted sequence listing (Name: 3338_046PC05_SL.txt, Size: 245,908 bytes; and Date of Creation: February 4, 2016) submitted in this application is incorporated herein by reference in its entirety.

FIELD OF INVENTION

[0003] The present invention relates to LNA oligonucleotides (oligomers) having two flanks, wherein one or both flanks comprise both LNA and DNA nucleosides.

BACKGROUND

[0004] Antisense oligonucleotides have been studied as potential drugs for diseases such as cancers (including lung cancer, colorectal carcinoma, pancreatic carcinoma, malignant glioma and malignant melanoma), diabetes, Amyotrophic lateral sclerosis (ALS), Duchenne muscular dystrophy and diseases such as asthma, arthritis and pouchitis with an inflammatory component. Antisense therapy can treat genetic disorders or infections when the genetic sequence of a particular gene is known to be associated with a particular disease. A strand of nucleic acid (DNA, RNA or a chemical analog) can bind to the

corresponding sequence (e.g. mRNA, pre-mRNA, etc.) and modulate the gene expression (e.g. effectively turn the gene "off").

[0005] While significant progress has been made, there remain challenges to antisense oligonucleotide development. One such challenge is off-target, non-specific binding of oligonucleotides, which can potentially lead to undesired toxicities in vivo. Therefore, there still is a need to develop improved oligomers with less toxicity and increased safety profiles.

SUMMARY OF INVENTION

[0006] The present invention is based on the discovery that a particular design of an oligomer (e.g., DNA nucleosides within the wing regions of LNA gapmer oligonucleotides) can reduce a potential off-target binding (e.g., toxicity) of the oligomer while retaining the affinity (e.g., potency) to its intended target.

[0007] The invention provides for an oligomer of at least 10 contiguous nucleotides in length, comprising region A, region B, and region C (A-B-C), wherein region B comprises at least 5 consecutive nucleoside units and is flanked at 5' by the A region of 1-8 contiguous nucleoside units and at 3' by the C region of 1-8 contiguous nucleoside units, wherein the B region, when formed in a duplex with a complementary RNA, is capable of recruiting RNaseH, and wherein region A and region C are selected from the group consisting of:

- (i) region A comprising a 5' LNA nucleoside unit, a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit, and region C comprising at least two 3' LNA nucleoside units;
- (ii) region A comprising at least one 5' LNA nucleoside unit, and region C comprising a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units; and
- (iii) region A comprising a 5' LNA nucleoside unit, a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit, and region C comprising a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units.

- [0008] Also provided is a conjugate comprising the oligomer described herein covalently attached to at least one non-nucleotide or non-polynucleotide moiety.
- [0009] Some embodiments of the application include a pharmaceutical composition comprising the oligomer or the conjugate and a pharmaceutically acceptable excipient, carrier, or diluent.
- [0010] The invention provides for the use of the oligomer or the conjugate of the invention as a medicament for treatment of a disease or condition associated with the target of the oligomer or the oligomer or the conjugate for use in treating a disease or condition associated with the target of the oligomer.
- [0011] In one embodiment, the invention provides for the oligomer or the conjugate of the invention as a medicament. In another embodiment, the invention provides for the oligomer or the conjugate of the invention for use in the treatment of a disease or condition.
- [0012] In other embodiments, the invention provides for a method for inhibiting the expression of a RNA in a target cell which is expressing said target, said method comprising administering an oligomer, or a conjugate or the pharmaceutical composition of the invention in an effective amount to the cell. In some embodiments the method is *in vivo*. In some embodiments the method is *in vitro*.
- [0013] In some embodiments, the oligomer or conjugate of the invention is administered via intracerebroventricular (ICV) administration. In some embodiments, the oligomer or conjugate of the invention is administered via intracerebral administration. In some embodiments, the oligomer or conjugate of the invention is administered via epidural administration. In some embodiments, the oligomer or conjugate of the invention is administered via intrathecal administration.
- [0014] The invention provides for an *in vivo* or *in vitro* method for modulating the expression of a RNA target cell which is expressing said target, the method comprising administering an oligomer, or a conjugate or the pharmaceutical composition of the invention in an effective amount to said cell.
- [0015] Certain embodiments of the application also include a method of reducing off-target binding of an oligomer comprising administering an oligomer disclosed herein, wherein the administering of the oligomer results in less hybridization to unintended targets compared to the administration of a corresponding oligomer with a different design (e.g.,

a design of a traditional gapmer (*e.g.* G-H-I described elsewhere herein or fully modified).

[0016] In some embodiments, the oligomer is from 10 to 50 nucleotides in length. In some embodiments, the oligomers of the invention comprise DNA and LNA nucleosides only, such as DNA and beta-D-oxy-nucleosides only. In some embodiments, the oligomers of the invention do not comprise a 2' substituted nucleoside. In some embodiments, the oligomers of the invention do not comprise a 2'-O-MOE nucleoside.

EMBODIMENTS

- [0017]** E1. An oligomer of at least 10 contiguous nucleotides in length, comprising region A, region B, and region C (A-B-C), wherein region B comprises at least 5 consecutive nucleoside units and is flanked at 5' by region A of 1-8 contiguous nucleoside units and at 3' by region C of 1-8 contiguous nucleoside units, wherein region B, when formed in a duplex with a complementary RNA, is capable of recruiting RNaseH, and wherein region A and region C are selected from the group consisting of:
- (i) region A comprises a 5' LNA nucleoside unit and a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit, and, region C comprises at least two 3' LNA nucleosides; or
 - (ii) region A comprises at least one 5' LNA nucleoside and region C comprises a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units, and
 - (iii) region A comprises a 5' LNA nucleoside unit and a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit; and region C comprises a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units.
- [0018]** E2. The oligomer according to embodiment 1, wherein region A or region C comprises 1, 2, or 3 DNA nucleoside units.
- [0019]** E3. The oligomer according to embodiment 1, wherein region A and region C comprise 1, 2, or 3 DNA nucleoside units.

- [0020] E4. The oligomer according to any one of embodiments 1 – 3, wherein region B comprises at least five consecutive DNA nucleoside units.
- [0021] E5. The oligomer according to embodiment 4, wherein region B comprises 6, 7, 8, 9, 10, 11, 12, 13 or 14 consecutive DNA nucleoside units.
- [0022] E6. The oligomer according to any one of embodiments 1 – 5, wherein region B is 8, 9, 10, 11, or 12 nucleotides in length.
- [0023] E7. The oligomer according to any one of embodiments 1 – 6, wherein region A comprises two 5' terminal LNA nucleoside units.
- [0024] E8. The oligomer according to any one of embodiments 1 – 7, wherein region A has formula 5'[LNA]₁₋₃[DNA]₁₋₃[LNA]₁₋₃, or 5'[LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂.
- [0025] E9. The oligomer according to any one of embodiments 1 – 8 wherein region C has formula [LNA]₁₋₃[DNA]₁₋₃[LNA]₂₋₃ 3', or [LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂[DNA]₁₋₂[LNA]₂₋₃ 3'.
- [0026] E10. The oligomer according to any one of embodiments 1 – 9, wherein region A has formula 5'[LNA]₁₋₃[DNA]₁₋₃[LNA]₁₋₃, or 5'[LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂, and region C comprises 2, 3, 4 or 5 consecutive LNA nucleoside units.
- [0027] E11. The oligomer according to any one of embodiments 1 – 9, wherein region C has formula [LNA]₁₋₃[DNA]₁₋₃[LNA]₂₋₃ 3' or [LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂[DNA]₁₋₂[LNA]₂₋₃ 3', and region A comprises 1, 2, 3, 4 or 5 consecutive LNA nucleoside units.
- [0028] E12. The oligomer according to any one of embodiments 1 – 11, wherein region A has a sequence of LNA and DNA nucleosides, 5' – 3' selected from the group consisting of L, LL, LDL, LLL, LLDL, LDLL, LDDL, LLLL, LLLLL, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDDL, LLLLLL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LDDDLL, LDDL, LDLLDL, LDLDLL, LDDDLL, LLDDL, and LDLDLD, wherein L represents a LNA nucleoside, and D represents a DNA nucleoside.
- [0029] E13. The oligomer according to any one of embodiments 1 – 12, wherein region C has a sequence of LNA and DNA nucleosides, 5' – 3' selected from the group

consisting of LL, LLL, LLLL, LDLL, LLLL, LLDLL, LDLLL, LDDLL, LDDLLL, LLDDLL, LDLDLL, LDDDLL, LDLDLL, LDDDLL, and LLDLDLL.

[0030] E14. The oligomer according to any one of embodiments 1 – 13, wherein region A has a sequence of LNA and DNA nucleosides, 5' – 3' selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLLDL, LLDLL, LDLLL, LLDDL, LDDLL, LLDLD, LDLLD, LDDDL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LLDDL, LDDL, LDLLD, LDLDL, LDDDL, LLDDL, and LDLDL, and region C has a sequence of LNA and DNA nucleosides, 5' – 3' selected from the group consisting of LDLL, LLLL, LLDLL, LDLLL, LDDL, LDDLLL, LLDDL, LDLDL, LDDDL, LDLDL, LDDL, LDDD, and LLDLD.

[illegible]

[illegible]

[0032] E16. The oligomer according to any one of embodiments 1 – 11, wherein the contiguous nucleotides comprise an alternating sequence of LNA and DNA nucleoside units, 5' – 3', selected from the group consisting of: **2-3-2-8-2, 1-1-2-1-1-9-2, 3-10-1-1-2, 3-9-1-2-2, 3-8-1-3-2, 3-8-1-1-1-1-2, 3-1-1-9-3, 3-1-1-8-1-1-2, 4-9-1-1-2, 4-8-1-2-2, 3-3-1-8-2, 3-2-1-9-2, 3-2-2-8-2, 3-2-2-7-3, 5-7-1-2-2, 1-1-3-10-2, 1-1-3-7-1-2-2,**

1-1-4-9-2, 2-1-3-9-2, 3-1-1-10-2, 3-1-1-7-1-2-2, 3-1-2-9-2, 4-7-1-3-2, 5-9-1-1-2, 4-10-1-1-2, 3-11-1-1-2, 2-1-1-10-1-1-2, 1-1-3-9-1-1-2, 3-10-1-2-2, 3-9-1-3-2, 3-8-1-1-1-2-2, 4-9-1-2-2, 4-9-1-1-3, 4-8-1-3-2, 4-8-1-2-3, 4-8-1-1-1-1-2, 4-7-1-2-1-1-2, 4-7-1-1-1-2-2, 2-1-2-11-2, 2-1-3-8-1-1-2, 3-1-1-11-2, 3-1-1-9-1-1-2, 3-1-1-8-1-2-2, 3-1-1-7-1-1-1-1-2, 4-9-2-1-2, 4-7-1-3-3, 5-9-1-1-3, 5-9-1-2-2, 4-10-2-1-2, 4-10-1-1-3, 4-10-1-2-2, 3-11-2-1-2, 3-11-1-1-3, 5-9-2-1-2, 3-11-1-2-2, 2-1-2-9-1-2-2, 3-1-1-10-1-1-2, 3-1-1-9-1-2-2, 4-9-1-1-1-1-2, 4-8-2-1-1-1-2, 1-1-3-10-2-1-2, 2-1-2-10-2-1-2, 2-1-1-12-4, 2-2-1-11-4, 3-1-1-11-4, 2-1-1-13-3, 2-1-2-11-4, 2-2-1-12-3, 3-11-1-2-3, 3-1-1-12-3, 2-1-2-12-3, 4-11-2-1-2, 4-10-2-2-2, 3-2-1-9-1-1-3, 2-2-1-1-1-9-4, 2-2-2-9-1-1-3, 3-1-1-9-1-1-1-1-2, 2-1-2-9-1-2-3, 3-1-1-10-1-1-3, 2-1-1-2-1-9-4, 4-9-1-1-1-2-2, 3-1-1-9-1-2-3, 2-1-1-1-1-10-4, 2-1-2-10-1-1-3, 2-1-1-1-1-9-2-1-2, 2-2-2-9-2-1-2, 4-9-1-2-1-1-2, 3-2-1-9-2-1-2, 2-1-2-9-2-2-2, 2-1-1-1-1-9-1-1-3, 3-1-1-9-2-2-2, 2-2-2-10-4, 2-1-2-9-1-1-1-1-2, 4-10-1-2-3, 3-2-1-10-4, 3-1-1-10-2-1-2, 4-10-1-1-1-1-2, 4-11-1-1-3, and 2-2-2-10-1-1-2; wherein the first numeral represents an number of LNA units, the next a number of DNA units, and alternating LNA and DNA regions thereafter.

- [0033] E17. The oligomer according to any one of embodiments 1 – 16, wherein the maximum number of contiguous LNA nucleosides in regions A and C is 2 or 3.
- [0034] E18. The oligomer according to any one of embodiments 1 – 17, wherein the maximum number of contiguous DNA nucleosides in regions A and C is 1 or 2.
- [0035] E19. The oligomer according to any one of embodiments 1-18, wherein the LNA nucleoside units present in regions A and C are beta-D LNA units, such as beta-D-oxy LNA nucleoside units or (S)cEt nucleoside LNA units.
- [0036] E20. The oligomer according to any one of embodiments 1-18, wherein the LNA nucleoside units present in regions A and C are beta-D-oxy LNA nucleoside units.
- [0037] E21. The oligomer according to any one of embodiments 1 – 20, wherein the oligomer comprises at least one phosphorothioate internucleoside linkage.
- [0038] E22. The oligomer according to any one of embodiments 1 – 20, wherein the internucleoside linkages within region B are phosphorothioate internucleoside linkages.
- [0039] E23. The oligomer according to any one of embodiments 1 – 20, wherein all the internucleoside linkages within regions A, B and C are phosphorothioate internucleoside linkages.

- [0040] E24. A conjugate comprising the oligomer according to any one 1-23 covalently attached to at least one non-nucleotide or non-polynucleotide moiety.
- [0041] E25. The conjugate according to embodiment 24, wherein the at least one non-nucleotide or non-polynucleotide moiety is selected from the group consisting of proteins, peptides, glycoproteins, antibodies, antibody binding domains, fatty acids, sterols, sugar residues, a GalNAc conjugate such as a trivalent GalNAc cluster, polymers, PEG, and two or more combinations thereof.
- [0042] E26. The conjugate according to embodiment 24 or 25, wherein the at least one non-nucleotide or non-polynucleotide moiety is covalently attached to the oligomer via a biocleavable linker, such as a region of 1,2, 3, 4 or 5 phosphodiester linked DNA nucleotides.
- [0043] E27. A pharmaceutical composition comprising the oligomer of any one of embodiments 1 – 22, or the conjugate of any one of embodiments 24 – 26, and a pharmaceutically acceptable excipient, carrier, or diluent.
- [0044] E28. Use of the oligomer of any one of embodiments 1 – 23, or the conjugate of any one of embodiments 24 – 26 for the manufacture of a medicament in treating a disease or condition.
- [0045] E29. An *in vivo* or *in vitro* method for modulating the expression of an RNA in a cell which expresses said RNA, said method comprising contacting an effective amount of the oligomer of any one of embodiments 1-23, the conjugate of any one of embodiments 24- 26 or the pharmaceutical composition of embodiment 27 with said cell.
- [0046] E30. A method of producing an oligomer having a reduced non-specific binding comprising synthesizing the oligomer of any one of embodiments 1 to 23.
- [0047] E31. A method of treating a disease or condition in a subject in need thereof while reducing the toxicity of the treatment comprising administering the oligomer of any one of embodiments 1-23, the conjugate of any one of embodiments 24- 26, or the pharmaceutical composition of embodiment 27 to the subject, wherein the administering treats the disease or condition while reducing the toxicity of the treatment compared to a treatment using a corresponding reference oligomer, which reference oligomer comprises formula G-H-I, wherein region H is identical to region B, and regions G and I comprise all LNA nucleoside units.

- [0048] E32. The oligomer of any one of embodiments 1 to 23 for use in treating a neurological disease or condition in a subject in need thereof, wherein calcium oscillations in neuronal cells that are in contact with the oligomer are about 70% or higher, about 75% or higher, about 80% or higher, about 85% or higher, about 90% or higher, about 95% or higher, about 96% or higher, about 97% or higher, about 98% or higher, about 99% or higher, about 100% or higher, about 120% or higher, about 140% or higher, about 160% or higher, about 180% or higher, about 200% or higher, about 220% or higher, about 240% or higher, or about 250% or higher compared to the calcium oscillations in vehicle control cells.
- [0049] E33. The oligomer of any one of embodiments 1 to 23 for use in treating a neurological disease or condition having the sequence score greater than or equal to 0.2, greater than or equal to 0.25, greater than or equal to 0.3, greater than or equal to 0.35, greater than or equal to 0.4, greater than or equal to 0.45, greater than or equal to 0.5, greater than or equal to 0.55, greater than or equal to 0.6, greater than or equal to 0.65, greater than or equal to 0.7, greater than or equal to 0.75, greater than or equal to 0.8, greater than or equal to 0.85, greater than or equal to 0.9, greater than or equal to 0.95, greater than or equal to 1.0, greater than or equal to 1.5, greater than or equal to 2.0, greater than or equal to 3.0, or greater than or equal to 4.0.

BRIEF DESCRIPTION OF FIGURES

- [0050] Figure 1 shows exemplary oligomers, designs (ASO Sequence), and chemical structure of the oligomers. Figure 1 lists the oligomer name, antisense oligomer (ASO) identification number, ASO sequence, SEQ ID Number, target start and end positions on the *MAPT* pre-mRNA sequence and chemical structure.
- [0051] Figure 2 shows exemplary oligomers targeting nucleotides 134,947 to 138,940 of SEQ ID NO: 1. Figure 2 lists the SEQ ID number, oligomer name, ASO identification number, ASO sequence, target start and end positions on the *MAPT* pre-mRNA sequence, target start on the mature mRNA sequence and normalized Tau/Tuj-1 and Tuj-1 immunocytochemistry values (as discussed in Example 2 below).
- [0052] Figure 3 shows the impact of Tau antisense oligonucleotides on spontaneous calcium oscillations in primary neurons and IC_{50} values of Tau neurons. Figure 3 lists the ASO identification number, ASO sequence, SEQ ID Number, target start and end

positions on the *MAPT* pre-mRNA sequence, calcium oscillation data as a percent of control (as discussed in Example 3 below) and IC₅₀ values of Tau neurons (as also discussed in Example 3 below).

[0053] Figure 4 shows the *in vivo* tolerability of exemplary oligomers and brain tau mRNA reduction. Figure 4 lists the ASO identification number, ASO sequence, SEQ ID Number, target start and end positions on the *MAPT* pre-mRNA sequence, *in vivo* acute tolerability score (as discussed in Example 5 below) and the percent of brain *MAPT* mRNA remaining after administration (as also discussed in Example 5 below).

[0054] Figure 5 shows the composition of the sequence of the selected oligomer and the sequence of Rho A which aligns with a portion of the MAPT genomic sequence (SEQ ID NO: 1). The RhoA sequence is listed as actttattccaaatacacttctt (SEQ ID NO: 267). The mismatches between the selected oligomers and the Rho A sequence were highlighted. The sequence of ASO-000757 has one mismatch compared to the corresponding RhoA sequence; The sequences of ASO-0001967, ASO-000755, and ASO-001941 have two mismatches compared to the corresponding RhoA sequence; and The sequences of ASO-000753, ASO-002038, ASO-001933, and ASO-001940 have four mismatches compared to the corresponding RhoA sequence. Figure 5 shows that the traditional gapmers (i.e., ASO-000757, ASO-000755, and ASO-000753) are not tolerated beyond 4 weeks following a single 100µg ICV bolus dose while the alternating flank gapmers exhibit tolerability beyond 4 weeks. Tubulin inhibition was highly correlated, in this data set, to long term tolerability. Rho A reduction greater than 25% (i.e., ASO-000757, ASO-000755, and ASO-000753) was also correlated with lack of long term tolerability (greater than 4 weeks following a single ICV bolus injection of 100µg of each ASO shown).

[0055] Figures 6A and 6B show exemplary oligomers, designs, and their chemical structures. Figure 6A lists the antisense oligomer (ASO) identification number, SEQ ID Number, ASO sequence, target start and end positions on the Tau pre-mRNA sequence, IC₅₀ values of Tau neurons (as discussed in Example 8 below) and percent Tau inhibition (as also discussed in Example 8 below). Figure 6B shows the specific chemical structure of the oligomers shown in Figure 6A and lists the antisense oligomer (ASO) identification number, ASO sequence, target start and end positions on the Tau pre-mRNA sequence and chemical structure.

- [0056] Figure 7 shows that an exemplary alternating flank oligomer, e.g., ASO-001933, produces dose responsive brain hTau protein reduction after a single ICV injection in hTau mouse brain. Saline or 50,100, 150 or 200µg of Tau ASO was injected ICV in hTau mice (n=10 per group). X-axis shows the doses of ASO-001933, and the Y-axis shows the hTau protein expression after the ASO injection compared to the hTau protein expression after the saline injection (% of saline).
- [0057] Figure 8A is an image of brain regions showing pathologic Tau accumulation in PSP.
- [0058] Figure 8B shows regional Tau mRNA changes in a control monkey (left) or in a monkey that had received two single 16mg intrathecal bolus doses of ASO-001933, one week apart (right). The Tau mRNA changes were assessed two weeks post-dosing by fluorescence in situ hybridization (FISH) using Tau mRNA specific probes in substantia nigra, pontine nucleus and central cerebellar dentate nucleus. Tau mRNA accumulation is shown as lighter shades.
- [0059] Figure 9A shows Tau protein reduction in brain following intrathecal dosing of ASO-001933 in nonhuman primates (NHPs). Regional Tau mRNA changes in a control monkey or a monkey that had received two single 8 mg intrathecal bolus doses of ASO-001933, two weeks apart, were assessed 4, 8, or 12 weeks post-dosing by Tau ELISAs (BT2/HT7 or Tau12/BT2). The regional Tau mRNA changes were measured in pons, cerebellum (CBL), parietal cortex (ParC), frontal cortex (FrC), occipital cortex (OccC), temporal cortex (TemC), and hippocampus (Hipp).
- [0060] Figure 9B shows Tau protein reduction in cerebrospinal fluid (CSF) following intrathecal dosing of ASO-001933 in nonhuman primates (NHPs). Y-axis shows percent baseline of Tau protein reduction in CSF, and X-axis shows weeks post last dose.
- [0061] Figure 10A shows that ASO-002038 (Tau ASO) produces durable, dose responsive brain hTau mRNA reduction after a single intracerebroventricular (ICV) injection in hTau mouse brain. Saline or 25, 50,100, and 150µg of Tau ASO was injected ICV in hTau mice (n=10 per group). The frontal cortical region was dissected 1 week post dose to determine total Tau mRNA levels by qRT-PCR. 1-way ANOVA analysis was used ***p<0.001. Error bars represent mean +/- SEM.
- [0062] Figure 10B shows that ASO-002038 (Tau ASO) produces durable, dose responsive brain hTau mRNA reduction after a single intrathecal (IT) injection in surgical lumbar

catheterized rats. Saline or 400, 900, and 1500 μ g of Tau ASO was injected IT in rats (n=10 per group). The frontal cortical region was dissected 1 week post dose to determine total Tau mRNA levels by qRT-PCR. 1-way ANOVA analysis was used ***p<0.001. Error bars represent mean +/- SEM.

[0063] Figures 11A and 11B show exemplary oligomers, designs, and chemical structures tested by QUANTIGENE[®] analysis. Figure 11A lists the antisense oligomer (ASO) identification number, SEQ ID Number, ASO sequence, target start and end positions on the Tau pre-mRNA sequence, start position on the mature mRNA sequence, and QUANTIGENE[®] expression of mRNA (as discussed in Example 10 below). Figure 11B shows the specific chemical structure of the oligomers shown in Figure 11A and lists the antisense oligomer (ASO) identification number, ASO sequence, target start and end positions on the Tau pre-mRNA sequence and chemical structure.

DETAILED DESCRIPTION OF INVENTION

I. Definitions

[0064] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0065] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0066] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0067] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular

Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

- [0068]** Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.
- [0069]** The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, *e.g.*, 10 percent, up or down (higher or lower). For example, if it is stated that "the oligomer reduces expression of the target protein in a cell following administration of the oligomer by at least about 60%," it is implied that the target protein levels are reduced by a range of 50% to 70%.
- [0070]** The term "nucleic acids" or "nucleotides" is intended to encompass plural nucleic acids. In some embodiments, the term "nucleic acids" or "nucleotides" refers to a target sequence, *e.g.*, pre-mRNAs, mRNAs, or DNAs *in vivo* or *in vitro*. When the term refers to the nucleic acids or nucleotides in a target sequence, the nucleic acids or nucleotides can be naturally occurring sequences within a cell. In other embodiments, "nucleic acids" or "nucleotides" refers to a sequence in the oligomers of the invention. When the term refers to a sequence in the oligomers, the nucleic acids or nucleotides are not naturally occurring. In one embodiment, the nucleic acids or nucleotides in the oligomers are produced synthetically or recombinantly, but are not a naturally occurring sequence or a fragment thereof. In another embodiment, the nucleic acids or nucleotides in the oligomers contain at least one nucleotide analog that is not naturally occurring in nature. The term "nucleic acid" or "nucleoside" refers to a single nucleic acid segment, *e.g.*, a

DNA, an RNA, or an analog thereof, present in a polynucleotide. "Nucleic acid" or "nucleoside" includes naturally occurring nucleic acids or non-naturally occurring nucleic acids. In some embodiments, the terms "nucleotide", "unit" and "monomer" are used interchangeably. It will be recognized that when referring to a sequence of nucleotides or monomers, what is referred to is the sequence of bases, such as A, T, G, C or U, and analogs thereof.

[0071] The term "nucleotide" as used herein, refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked group (linkage group), such as a phosphate or phosphorothioate internucleotide linkage group, and covers both naturally occurring nucleotides, such as DNA or RNA, and non-naturally occurring nucleotides comprising modified sugar and/or base moieties, which are also referred to as "nucleotide analogs" herein. Herein, a single nucleotide (unit) can also be referred to as a monomer or nucleic acid unit. In certain embodiments, the term "nucleotide analogs" refers to nucleotides having modified sugar moieties. Non-limiting examples of the nucleotides having modified sugar moieties (e.g., LNA) are disclosed elsewhere herein, *e.g.*, 2'-O-methyl, 2'-fluoro (2'-F), 2'-O-methoxyethyl (2'-MOE), and 2',4'-constrained 2'-O-ethyl (cEt). In other embodiments, the term "nucleotide analogs" refers to nucleotides having modified base moieties. The nucleotides having modified base moieties include, but are not limited to, 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

[0072] The term "nucleoside" as used herein is used to refer to a glycoside comprising a sugar moiety and a base moiety, and can therefore be used when referring to the nucleotide units, which are covalently linked by the internucleotide linkages between the nucleotides of the oligomer. In the field of biotechnology, the term "nucleotide" is often used to refer to a nucleic acid monomer or unit, and as such in the context of an oligonucleotide can refer to the base – such as the "nucleotide sequence", typically refer to the nucleobase sequence (*i.e.* the presence of the sugar backbone and internucleoside linkages are implicit). Likewise, particularly in the case of oligonucleotides where one or more of the internucleoside linkage groups are modified, the term "nucleotide" can refer to a "nucleoside" for example the term "nucleotide" can be used, even when specifying the presence or nature of the linkages between the nucleosides.

- [0073] The term "nucleotide length" as used herein means the total number of the nucleotides (monomers) in a given sequence. For example, the sequence of AtTTCcaaattcactTTtAC (SEQ ID NO: 91) has 20 nucleotides; thus the nucleotide length of the sequence is 20. The term "nucleotide length" is therefore used herein interchangeably with "nucleotide number."
- [0074] As one of ordinary skill in the art would recognize, the 5' terminal nucleotide of an oligonucleotide does not comprise a 5' internucleotide linkage group, although it may or may not comprise a 5' terminal group.
- [0075] As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, untranslated regions ("UTRs"), and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide.
- [0076] The term "non-coding region" as used herein means a nucleotide sequence that is not a coding region. Examples of non-coding regions include, but are not limited to, promoters, ribosome binding sites, transcriptional terminators, introns, untranslated regions ("UTRs"), non-coding exons and the like. Some of the exons can be wholly or part of the 5' untranslated region (5' UTR) or the 3' untranslated region (3' UTR) of each transcript. The untranslated regions are important for efficient translation of the transcript and for controlling the rate of translation and half-life of the transcript.
- [0077] The term "region" when used in the context of a nucleotide sequence refers to a section of that sequence. For example, the phrase "region within a nucleotide sequence" or "region within the complement of a nucleotide sequence" refers to a sequence shorter than the nucleotide sequence, but longer than at least 10 nucleotides located within the particular nucleotide sequence or the complement of the nucleotides sequence, respectively. The term "sub-sequence" or "subsequence" can also refer to a region of a nucleotide sequence.

- [0078] The term "downstream," when referring to a nucleotide sequence, means that a nucleic acid or a nucleotide sequence is located 3' to a reference nucleotide sequence. In certain embodiments, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.
- [0079] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence.
- [0080] As used herein, the term "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions can include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites, UTRs, and stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.
- [0081] The term "transcript" as used herein can refer to a primary transcript that is synthesized by transcription of DNA and becomes a messenger RNA (mRNA) after processing, *i.e.*, a precursor messenger RNA (pre-mRNA), and the processed mRNA itself. The term "transcript" can be interchangeably used with "pre-mRNA" and "mRNA." After DNA strands are transcribed to primary transcripts, the newly synthesized primary transcripts are modified in several ways to be converted to their mature, functional forms to produce different proteins and RNAs such as mRNA, tRNA, rRNA, lncRNA, miRNA and others. Thus, the term "transcript" can include exons, introns, 5' UTRs, and 3' UTRs.
- [0082] The term "expression" as used herein refers to a process by which a polynucleotide produces a gene product, for example, a RNA or a polypeptide. It includes, without limitation, transcription of the polynucleotide into messenger RNA (mRNA) and the translation of an mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational

modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0083] The terms "identical" or percent "identity" in the context of two or more nucleic acids refer to two or more sequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software are known in the art that can be used to obtain alignments of amino acid or nucleotide sequences.

[0084] One such non-limiting example of a sequence alignment algorithm is the algorithm described in Karlin *et al.*, 1990, *Proc. Natl. Acad. Sci.*, 87:2264-2268, as modified in Karlin *et al.*, 1993, *Proc. Natl. Acad. Sci.*, 90:5873-5877, and incorporated into the NBLAST and XBLAST programs (Altschul *et al.*, 1991, *Nucleic Acids Res.*, 25:3389-3402). In certain embodiments, Gapped BLAST can be used as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. BLAST-2, WU-BLAST-2 (Altschul *et al.*, 1996, *Methods in Enzymology*, 266:460-480), ALIGN, ALIGN-2 (Genentech, South San Francisco, California) or Megalign (DNASTAR) are additional publicly available software programs that can be used to align sequences. In certain embodiments, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (*e.g.*, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 90 and a length weight of 1, 2, 3, 4, 5, or 6). In certain alternative embodiments, the GAP program in the GCG software package, which incorporates the algorithm of Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) can be used to determine the percent identity between two amino acid sequences (*e.g.*, using either a BLOSUM 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5). Alternatively, in certain embodiments, the percent identity between nucleotide or amino acid sequences is determined using the algorithm of Myers and Miller (CABIOS, 4:11-17 (1989)). For example, the percent identity can be determined using the ALIGN program (version 2.0) and using a PAM120 with residue table, a gap length penalty of 12 and a gap penalty of 4. One skilled in the art can determine appropriate parameters for maximal alignment by particular alignment

software. In certain embodiments, the default parameters of the alignment software are used.

- [0085]** In certain embodiments, the percentage identity "X" of a first nucleotide sequence to a second nucleotide sequence is calculated as $100 \times (Y/Z)$, where Y is the number of amino acid residues scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.
- [0086]** Different regions within a single polynucleotide target sequence that align with a polynucleotide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.
- [0087]** As used herein, the terms "homologous" and "homology" are interchangeable with the terms "identity" and "identical."
- [0088]** In determining the degree of "complementarity" between oligomers of the invention (or regions thereof) and the target region of the target nucleic acid, the degree of "complementarity" (also, "homology" or "identity") is expressed as the percentage identity (or percentage homology) between the sequence of the oligomer (or region thereof) and the sequence of the target region (or the reverse complement of the target region) that best aligns therewith. The percentage is calculated by counting the number of aligned bases that are identical between the two sequences, dividing by the total number of contiguous monomers in the oligomer, and multiplying by 100. In such a comparison, if gaps exist, it is preferable that such gaps are merely mismatches rather than areas where the number of monomers within the gap differs between the oligomer of the invention and the target region.
- [0089]** The term "complement" as used herein indicates a sequence that is complementary to a reference sequence. It is well known that complementarity is the base principle of DNA replication and transcription as it is a property shared between two DNA or RNA

sequences, such that when they are aligned antiparallel to each other, the nucleotide bases at each position in the sequences will be complementary, much like looking in the mirror and seeing the reverse of things. Therefore, for example, the complement of a sequence of 5'"ATGC"3' can be written as 3'"TACG"5' or 5'"GCAT"3'. The terms "reverse complement", "reverse complementary" and "reverse complementarity" as used herein are interchangeable with the terms "complement", "complementary" and "complementarity."

[0090] The term "design" or "oligomer design" or "ASO Sequence" as used herein refers to a pattern of nucleotides (e.g., DNA) and nucleotide analogs (e.g., LNA) in a given sequence. As used herein, the design of an oligomer is shown by a combination of upper case letters and lower case letters. For example, an oligomer sequence of tatttccaaattcactttta (SEQ ID NO: 337) can have oligomer designs of ASO-002350 (TA_tTT_ccaaattcactTTTA) (SEQ ID NO: 141), ASO-002374 (TA_tTT_ccaaattcacTtTTA) (SEQ ID NO: 142), ASO-002386 (TATT_tccaaattcaCTtTA) (SEQ ID NO: 143), ASO-002227 (TAT_tT_ccaaattcactTTTA) (SEQ ID NO: 144), ASO-002245 (TAt_tTC_caaattcactTTTA) (SEQ ID NO: 145), ASO-002261 (TAT_tT_ccaaattcacTTtTA) (SEQ ID NO: 146), ASO-002276 (AT_ttC_caaattcactTTTA) (SEQ ID NO: 147), ASO-002228 (TATT_tccaaattcaCtTtTA) (SEQ ID NO: 148), ASO-002255 (TATT_tccaaattcactTTTA) (SEQ ID NO: 338), ASO-002285 (TATT_tccaaattcacTTtTA) (SEQ ID NO: 149), ASO-002230 (TATT_tccaaattcacTtTTA) (SEQ ID NO: 150), ASO-002256 (TATT_tccaaattcAcTtTA) (SEQ ID NO: 151), or ASO-002279 (TATT_tccaaattcActTtTA) (SEQ ID NO: 152), wherein the upper case letter indicates a nucleotide analog (e.g., LNA) and the lower case letter indicates a nucleotide (e.g., DNA).

[0091] The term "chemical structure" of an oligomer as used herein refers to a detailed description of the components of the oligomers, e.g., nucleotides (e.g., DNA), nucleotide analogs (e.g., beta-D-oxy-LNA), nucleotide base (e.g., A, T, G, C, U, or MC), and backbone structure (e.g., phosphorothioate or phosphodiester). For example, a chemical structure of ASO-002350 can be OxyTs OxyAs DNAts OxyTs OxyTs DNACs DNACs DNAas DNAas DNAas DNAts DNAts DNACs DNAas DNACs DNAts OxyTs OxyTs OxyTs OxyAs. Figures 2, 16B, and 20B lists non-limiting examples of chemical structures that can be applied to any one of the oligomers disclosed herein.

[0092] "Potency" is normally expressed as an IC₅₀ or EC₅₀ value, in μM, nM or pM unless otherwise stated. Potency can also be expressed in terms of percent inhibition. IC₅₀ is the

median inhibitory concentration of a therapeutic molecule. EC50 is the median effective concentration of a therapeutic molecule relative to a vehicle or saline control. In functional assays, IC50 is the concentration that reduces a biological response, e.g., transcription of mRNA or protein expression, by 50% of the biological response that is achieved without the therapeutic molecule. In functional assays, EC50 is the concentration of a therapeutic molecule that produces 50% of the biological response, e.g., transcription of mRNA or protein expression. IC50 or EC50 can be calculated by any number of means known in the art.

- [0093] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, sports animals, and zoo animals including, *e.g.*, humans, non-human primates, dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, bears, and so on.
- [0094] The term "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition would be administered. Such composition can be sterile.
- [0095] An "effective amount" of an oligomer as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and in a routine manner, in relation to the stated purpose.
- [0096] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and (2) prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In certain embodiments, a subject is successfully "treated" for a disease or condition disclosed elsewhere herein according to the methods provided herein if the patient shows, *e.g.*, total, partial, or transient alleviation or elimination of symptoms associated with the disease or disorder.

II. The Oligomer

- [0097] The present invention employs oligomeric compounds (referred to herein as oligomers) having an improved oligomer design. The oligomer design is improved such that the design provides at least one enhanced property. For example, while not bound by any theory, the oligomers of the present disclosure have a reduced non-specific binding, but retain the affinity to the target nucleic acid. The reduced non-specific binding of the oligomer can result in less toxicity of the oligomers, especially when administered *in vivo*. Therefore, the oligomers can be used in modulating the function of nucleic acid molecules *in vivo*. The term "oligomer" in the context of the present invention, refers to a molecule formed by covalent linkage of two or more nucleotides (*i.e.*, an oligonucleotide).
- [0098] The oligomer comprises a contiguous nucleotide sequence of from about 10 to about 50, such as 10–20, 16–20, 15–25, 10–30, 10–35, 10–40, or 10–45 nucleotides in length. The terms "antisense oligomer," "antisense oligonucleotide," and "ASO" as used herein are interchangeable with the term "oligomer."
- [0099] A reference to a SEQ ID number includes a particular sequence, but does not include an oligomer design as shown in Figure 1, 6B, and 11B. Furthermore, the oligomers disclosed in the figures herein show a representative design, but are not limited to the specific design shown in the tables. Herein, a single nucleotide (unit) can also be referred to as a monomer or unit. When this specification refers to a specific ASO number (or oligomer name), the reference includes the specific oligomer design. For example, when a claim (or this specification) recites SEQ ID NO: 241, it includes the nucleotide sequence of actttatttccaaattcacttttac. When a claim (or the specification) recites ASO-002019, it includes the nucleotide sequence of actttatttccaaattcacttttac with the oligomer design shown in the figures (*i.e.*, ActtTatttccaaattcactTTtaC). Alternatively, ASO-002019 can be written as ActtTatttccaaattcactTTtaC, wherein the upper case letter is a modified nucleotide (*e.g.*, LNA) and the lower case letter is a non-modified nucleotide (*e.g.*, DNA). ASO-002019 can also be written as SEQ ID NO: 241, wherein each of the first nucleotide, the fifth nucleotide, the 21st nucleotide, the 22nd nucleotide, and the 25th nucleotide from the 5' end is a modified nucleotide, *e.g.*, LNA, and each of the other nucleotides is a non-modified nucleotide (*e.g.*, DNA). The oligomers of the invention can also be written as SEQ ID NO: 241 with the chemical structure shown in Figure 1, *i.e.*,

OxyAs OxyMCs DNAts DNAts OxyTs DNAas DNAts DNAts DNAts DNacs DNacs
DNAas DNAas DNAas DNAts DNAts DNacs DNAas DNacs DNAts OxyTs OxyTs
DNAts DNAas OxyMC.

[0100] In various embodiments, the oligomer of the invention does not comprise RNA (units). In some embodiments, the oligomer comprises one or more DNA units. In one embodiment, the oligomer according to the invention is a linear molecule or is synthesized as a linear molecule. In some embodiments, the oligomer is a single stranded molecule, and does not comprise short regions of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to equivalent regions within the same oligomer (*i.e.* duplexes) - in this regard, the oligomer is not (essentially) double stranded. In some embodiments, the oligomer is essentially not double stranded. In some embodiments, the oligomer is not a siRNA. In various embodiments, the oligomer of the invention can consist entirely of the contiguous nucleotide region. Thus, in some embodiments the oligomer is not substantially self-complementary

[0101] In certain embodiments, the oligomers of the invention can be used in treating a neurological disease or condition in a subject in need thereof, wherein calcium oscillations in neuronal cells that are in contact with the oligomer are about 70% or higher, about 75% or higher, about 80% or higher, about 85% or higher, about 90% or higher, about 95% or higher, about 96% or higher, about 97% or higher, about 98% or higher, about 99% or higher, about 100% or higher, about 120% or higher, about 140% or higher, about 160% or higher, about 180% or higher, about 200% or higher, about 220% or higher, about 240% or higher, or about 250% or higher compared to the calcium oscillations in vehicle control cells.

[0102] In other embodiments, the oligomers of the invention can be used in treating a neurological disease or condition, wherein the oligomer has a sequence score greater than or equal to 0.2, greater than or equal to 0.25, greater than or equal to 0.3, greater than or equal to 0.35, greater than or equal to 0.4, greater than or equal to 0.45, greater than or equal to 0.5, greater than or equal to 0.55, greater than or equal to 0.6, greater than or equal to 0.65, greater than or equal to 0.7, greater than or equal to 0.75, greater than or equal to 0.8, greater than or equal to 0.85, greater than or equal to 0.9, greater than or equal to 0.95, greater than or equal to 1.0, greater than or equal to 1.5, greater than or equal to 2.0, greater than or equal to 3.0, or greater than or equal to 4.0.

II.A. The Target

[0103] A target nucleic acid is an intended target of the oligomer of the invention, and may for example, be a gene, a RNA, a mRNA, and pre-mRNA, a mature mRNA or a cDNA sequence. For *in vivo* or *in vitro* application, the oligonucleotide of the invention is typically capable of modulating the expression of the intended target nucleic acid in a cell which is expressing the target nucleic acid. The contiguous sequence of nucleobases of the oligomers is typically complementary to the target nucleic acid, as measured across the length of the oligonucleotide, optionally with the exception of one or two mismatches, and optionally excluding nucleotide based linker regions which may link the oligonucleotide to an optional functional group such as a conjugate. The target nucleic acid may, in some embodiments, be a RNA or DNA that is associated with any disease or condition. In some embodiments, the target nucleic acid may be RNA, such as a messenger RNA (e.g., a mature mRNA or a pre-mRNA).

[0104] In some embodiments, the oligomer of the invention is capable of hybridizing to the target nucleic acid under physiological condition, *i.e.*, *in vivo* condition. In some embodiments, the oligomer of the invention is capable of hybridizing to the target nucleic acid *in vitro*. In some embodiments, the oligomer of the invention is capable of hybridizing to the target nucleic acid *in vitro* under stringent conditions. Stringency conditions for hybridization *in vitro* are dependent on, *inter alia*, productive cell uptake, RNA accessibility, temperature, free energy of association, salt concentration, and time (*see, e.g.*, Stanley T Crooks, Antisense Drug Technology: Principles, Strategies and Applications, 2nd Edition, CRC Press (2007))). Generally, conditions of high to moderate stringency are used for *in vitro* hybridization to enable hybridization between substantially similar nucleic acids, but not between dissimilar nucleic acids. An example of stringent hybridization conditions include hybridization in 5X saline-sodium citrate (SSC) buffer (0.75 M sodium chloride/0.075 M sodium citrate) for 1 hour at 40° C, followed by washing the sample 10 times in 1X SSC at 40° C and 5 times in 1X SSC buffer at room temperature. *In vivo* hybridization conditions consist of intracellular conditions (*e.g.*, physiological pH and intracellular ionic conditions) that govern the hybridization of antisense oligonucleotides with target sequences. *In vivo conditions* can be mimicked *in vitro* by relatively low stringency conditions. For example, hybridization can be carried out *in vitro* in 2X SSC (0.3 M sodium chloride/0.03 M sodium citrate),

0.1% SDS at 37°C. A wash solution containing 4X SSC, 0.1% SDS can be used at 37° C, with a final wash in 1X SSC at 45° C.

[0105] In some embodiments, the oligomers of the invention bind to the target nucleic acid sequence and inhibit or reduce expression of the target nucleic acid transcript by at least 10% or 20% compared to the normal (*i.e.*, control) expression level in the cell, *e.g.*, at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% compared to the normal expression level (such as the expression level in the absence of the oligomer(s) or conjugate(s)) in the cell.

[0106] In certain embodiments, the oligomers of the invention bind to the target nucleic acid transcript and inhibit or reduce expression of the target nucleic acid mRNA by at least about 10% or about 20% compared to the normal (*i.e.* control) expression level in the cell, *e.g.*, at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or about 95% compared to the normal expression level (such as the expression level in the absence of the oligomer(s) or conjugate(s)) in the cell. In certain embodiments, the oligomer reduces expression of target protein in a cell following administration of the oligomer by at least 60%, at least 70%, at least 80%, or at least 90% compared to a cell not exposed to the oligomer (*i.e.*, control). In some embodiments, the oligomer reduces expression of target protein in a cell following administration of the oligomer by at least about 60%, at least about 70%, at least about 80%, or at least about 90% compared to a cell not exposed to the oligomer (*i.e.*, control).

[0107] In certain embodiments, the oligomer of the invention has at least one property selected from: (1) reduces expression of target mRNA in a cell, compared to a control cell that has not been exposed to the oligomer; (2) does not significantly reduce calcium oscillations in a cell; (3) does not significantly reduce tubulin intensity in a cell; (4) reduces expression of Tau target protein in a cell; and (5) any combinations thereof compared to a control cell that has not been exposed to the oligomer.

[0108] Calcium oscillations are important for the proper functions of neuronal cells. Networks of cortical neurons have been shown to undergo spontaneous calcium oscillations resulting in the release of the neurotransmitter glutamate. Calcium oscillations can also regulate interactions of neurons with associate glia, in addition to other associated neurons in the network, to release other neurotransmitters in addition to glutamate. Regulated calcium oscillations are required for homeostasis of neuronal

networks for normal brain function. (See, Shashank *et al.*, *Brain Research*, 1006(1): 8–17 (2004); Rose *et al.*, *Nature Neurosci.*, 4:773 – 774 (2001); Zonta *et al.*, *J Physiol Paris.*, 96(3-4):193-8 (2002); Pasti *et al.*, *J. Neurosci.*, 21(2): 477-484 (2001).) Glutamate also activates two distinct ion channels, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and N-methyl-D-aspartate (NMDA) receptors.

[0109] In some embodiments, the calcium oscillations measured in the present methods are AMPA-dependent calcium oscillations. In some embodiments, the calcium oscillations are NMDA-dependent calcium oscillations. In some embodiments, the calcium oscillations are gamma-aminobutyric acid (GABA)-dependent calcium oscillations. In some embodiments, the calcium oscillations can be a combination of two or more of AMPA-dependent, NMDA-dependent or GABA-dependent calcium oscillations.

[0110] In certain embodiments, the calcium oscillations measured in the present methods are AMPA-dependent calcium oscillations. In order to measure AMPA-dependent calcium oscillations, the calcium oscillations can be measured in the presence of Mg^{2+} ions (e.g., $MgCl_2$). In certain embodiments, the method further comprises adding Mg^{2+} ions (e.g., $MgCl_2$) at an amount that allows for detection of AMPA-dependent calcium oscillations. In some embodiments, the effective ion concentration amount allowing for detection of AMPA-dependent calcium oscillations is at least about 0.5 mM. In other embodiments, the effective ion concentration amount to induce AMPA-dependent calcium oscillations is at least about 0.6 mM, at least about 0.7 mM, at least about 0.8 mM, at least about 0.9 mM, at least about 1 mM, at least about 1.5 mM, at least about 2.0 mM, at least about 2.5 mM, at least about 3.0 mM, at least about 4 mM, at least about 5 mM, at least about 6 mM, at least about 7 mM, at least about 8 mM, at least about 9 mM, or at least about 10mM. In a particular embodiment, the concentration of Mg^{2+} ions (e.g., $MgCl_2$) useful for the methods is 1mM. In certain embodiments, the concentration of Mg^{2+} ions (e.g., $MgCl_2$) useful for the present methods is about 1 mM to about 10 mM, about 1 mM to about 15mM, about 1 mM to about 20 mM, or about 1 mM to about 25 mM. Mg^{2+} ions can be added by the addition of magnesium salts, such as magnesium carbonate, magnesium chloride, magnesium citrate, magnesium hydroxide, magnesium oxide, magnesium sulfate, and magnesium sulfate heptahydrate.

[0111] In some embodiments, calcium oscillations are measured in the present method through the use of fluorescent probes which detect the fluctuations of intracellular

calcium levels. For example, detection of intracellular calcium flux can be achieved by staining the cells with fluorescent dyes which bind to calcium ions (known as fluorescent calcium indicators) with a resultant, detectable change in fluorescence (*e.g.*, Fluo-4 AM and Fura Red AM dyes available from Molecular Probes, Eugene, OR, United States of America).

[0112] In other embodiments, the oligomers of the invention do not significantly reduce the tubulin intensity in a cell. In some embodiments, tubulin intensity is greater than or equal to 95%, greater than or equal to 90%, greater than or equal to 85%, greater than or equal to 80%, greater than or equal to 75%, or greater than or equal to 70% of tubulin intensity in a cell not exposed to the oligomer (or exposed to saline).

[0113] In some embodiments, such property is observed when using from 0.04nM to 400 μ M, concentration of the oligomer of the invention. In the same or a different embodiment, the inhibition or reduction of expression of target mRNA and/or target protein in the cell results in less than 100%, such as less than 98%, less than 95%, less than 90%, less than 80%, such as less than 70%, mRNA or protein levels compared to cells not exposed to the oligomer. Modulation of expression level can be determined by measuring target protein levels, *e.g.*, by methods such as SDS-PAGE followed by western blotting using suitable antibodies raised against the target protein. Alternatively, modulation of expression levels can be determined by measuring levels of mRNA, *e.g.*, by northern blot or quantitative RT-PCR. When measuring inhibition via mRNA levels, the level of down-regulation when using an appropriate dosage, such as from about 0.04nM to about 400 μ M concentration, is, in some embodiments, typically to a level of from about 10-20% the normal levels in the cell in the absence of the oligomer of the invention.

[0114] In certain embodiments, the oligomer of the invention has an *in vivo* tolerability less than or equal to a total score of 4, wherein the total score is the sum of a unit score of five categories, which are 1) hyperactivity; 2) decreased activity and arousal; 3) motor dysfunction and/or ataxia; 4) abnormal posture and breathing; and 5) tremor and/or convulsions, and wherein the unit score for each category is measured on a scale of 0-4. In certain embodiments, the *in vivo* tolerability is less than or equal to the total score of 3, the total score of 2, the total score of 1, or the total score of 0. In some embodiment, the assessment for *in vivo* tolerability is determined as described in Example 1 below.

[0115] In some embodiments, the oligomer can tolerate 1, 2, 3, or 4 (or more) mismatches, when hybridizing to the target sequence and still sufficiently bind to the target to show the desired effect, *i.e.*, down-regulation of the target mRNA and/or protein. Mismatches may, for example, be compensated by increased length of the oligomer nucleotide sequence and/or an increased number of nucleotide analogs, which are disclosed elsewhere herein.

[0116] In some embodiments, the oligomer of the invention comprises no more than 3 mismatches when hybridizing to the target sequence. In other embodiments, the contiguous nucleotide sequence comprises no more than 2 mismatches when hybridizing to the target sequence. In other embodiments, the contiguous nucleotide sequence comprises no more than 1 mismatch when hybridizing to the target sequence. In some embodiments the region within the complement or the region can consist of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 contiguous nucleotides, such as from 12-22, such as from 14-21 nucleotides. Suitably, in some embodiments, the region is of the same length as the contiguous nucleotide sequence of the oligomer of the invention.

[0117] However, it is recognized that, in some embodiments, the nucleotide sequence of the oligomer can comprise additional 5' or 3' nucleotides, such as, independently, 1, 2, 3, 4 or 5 additional nucleotides 5' and/or 3', which are non-complementary to the target sequence. In this respect the oligomer of the invention, can, in some embodiments, comprise a contiguous nucleotide sequence which is flanked 5' and/or 3' by additional nucleotides.

[0118] In some embodiments, the oligomer of the invention has a sequence score greater than or equal to 0.2, wherein the sequence score is calculated by formula I:

$$\frac{\text{\# of C nucleotides and analogs thereof} - \text{\# of G nucleotides and analogs thereof}}{\text{Total nucleotide length (number)}} \quad (\text{I})$$

[0119] In some embodiments, the method comprises measuring a sequence calculated by formula (IA):

$$\frac{\text{\# of C nucleotides and 5-methylcytosine nucleotides} - \text{\# of G nucleotides}}{\text{Total nucleotide length}} \quad (\text{IA})$$

In other embodiments, the oligomer of the invention has a sequence score greater than or equal to 0.2.

[0120] In these embodiments, a sequence score of greater than or equal to a cut off value corresponds to a reduced neurotoxicity of the oligomer. A sequence score of greater than or equal to a cut off value corresponds to a reduced neurotoxicity of the oligomer.

[0121] In certain embodiment, the oligomer of the invention has a sequence score greater than or equal to about 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0. In all of these embodiments, when the sequence score is greater than or equal to the cut off value, the oligomer is considered to have reduced neurotoxicity.

II.B. Oligomer Length

[0122] In some embodiments, the oligomers can comprise a contiguous nucleotide sequence of a total of 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, or 50 contiguous nucleotides in length.

[0123] In some embodiments, the oligomers comprise a contiguous nucleotide sequence of a total of about 10–22, such as 10–21 or 12–18, such as 13–17 or 12–16, such as 13, 14, 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides in length.

[0124] In some embodiments, the oligomers comprise a contiguous nucleotide sequence of a total of 10, 11, 12, 13, or 14 contiguous nucleotides in length.

[0125] In some embodiments, the oligomer according to the invention consists of no more than 22 nucleotides, such as no more than 21 or 20 nucleotides, such as no more than 18 nucleotides, such as 15, 16 or 17 nucleotides. In some embodiments the oligomer of the invention comprises less than 22 nucleotides. It should be understood that when a range is given for an oligomer, or contiguous nucleotide sequence length, the range includes the lower and upper lengths provided in the range, for example from (or between) 10–50, includes both 10 and 50.

II.C. Nucleosides and Nucleoside analogs

[0126] In one aspect of the invention, the oligomers comprise one or more non-naturally occurring nucleotide analogs. "Nucleotide analogs" as used herein are variants of natural nucleotides, such as DNA or RNA nucleotides, by virtue of modifications in the sugar and/or base moieties. Analogs could in principle be merely "silent" or "equivalent" to the natural nucleotides in the context of the oligonucleotide, *i.e.* have no functional effect on

the way the oligonucleotide works to inhibit target gene expression. Such "equivalent" analogs can nevertheless be useful if, for example, they are easier or cheaper to manufacture, or are more stable to storage or manufacturing conditions, or represent a tag or label. In some embodiments, however, the analogs will have a functional effect on the way in which the oligomer works to inhibit expression; for example by producing increased binding affinity to the target and/or increased resistance to intracellular nucleases and/or increased ease of transport into the cell. Specific examples of nucleoside analogs are described by *e.g.* Freier & Altmann; *Nucl. Acid Res.*, 1997, 25, 4429-4443 and Uhlmann; *Curr. Opinion in Drug Development*, 2000, 3(2), 293-213.

- [0127] The oligomers of the invention comprise at least two LNA nucleosides such as at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten LNA nucleosides. In some embodiments, the oligomer includes four, six, eight, or ten LNA nucleosides.
- [0128] In some embodiments, the oligomer of the invention comprises at least one "cEt" or "constrained ethyl" means a bicyclic nucleoside having a sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-CH(CH₃)-O-2'.
- [0129] "2'-O-methoxyethyl" (also 2'-MOE and 2'-O(CH₂)₂-OCH₃ 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.
- [0130] As used herein, "2'-F" refers to modification of the 2' position of the furanosyl sugar ring to comprise a fluoro group.
- [0131] As used herein, "2'-OMe" or "2'-OCH₃" or "2'-O-methyl" each refers to modification at the 2' position of the furanosyl sugar ring to comprise a -OCH₃ group.
- [0132] The oligomer can thus comprise a simple sequence of natural occurring nucleotides – for example, 2'-deoxynucleotides (referred to herein generally as "DNA"), but also possibly ribonucleotides (referred to herein generally as "RNA"), or a combination of such naturally occurring nucleotides and one or more non-naturally occurring nucleotides, *i.e.* nucleotide analogs. Such nucleotide analogs can suitably enhance the affinity of the oligomer for the target sequence.
- [0133] Examples of suitable nucleotide analogs are provided by WO2007/031091, which is incorporated by reference in its entirety, or are referenced therein.

- [0134] Incorporation of affinity-enhancing nucleotide analogs in the oligomer, such as LNA or 2'-substituted sugars, can allow the size of the specifically binding oligomer to be reduced, and can also reduce the upper limit to the size of the oligomer before non-specific or aberrant binding takes place.
- [0135] In some embodiments, the oligomer comprises at least one LNA. Additional details of the LNA compound are disclosed elsewhere herein. In some embodiments the oligomer comprises at least 2 LNAs. In some embodiments, the oligomer comprises from 3-10 LNAs, *e.g.*, 6 or 7 LNAs, *e.g.*, at least 3 or at least 4, or at least 5, or at least 6, or at least 7, or 8 LNAs. In some embodiments all the nucleotides analogs can be LNA.
- [0136] In a specific embodiment, the oligomer of the invention includes a bicyclic sugar. Non-limiting examples of the bicyclic sugar includes cEt, 2',4'-constrained 2'-O-methoxyethyl (cMOE), LNA, α -LNA, β -LNA, 2'-O,4'-C-ethylene-bridged nucleic acids (ENA), amino-LNA, oxy-LNA, or thio-LNA.
- [0137] The term "thio-LNA" comprises a locked nucleotide in which Y in general Formula III below is selected from S or -CH₂-S-. Thio-LNA can be in both beta-D and alpha-L-configuration.
- [0138] The term "amino-LNA" comprises a locked nucleotide in which Y in general Formula III below is selected from -N(H)-, N(R)-, CH₂-N(H)-, and -CH₂-N(R)- where R is selected from hydrogen and C₁₋₄-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.
- [0139] The term "oxy-LNA" comprises a locked nucleotide in which Y in general Formula III below represents -O-. Oxy-LNA can be in both beta-D and alpha-L-configuration.
- [0140] The term "ENA" comprises a locked nucleotide in which Y in general Formula III below is -CH₂-O- (where the oxygen atom of -CH₂-O- is attached to the 2'-position relative to the base B). R^c is hydrogen or methyl.
- [0141] In some exemplary embodiments LNA is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA and beta-D-thio-LNA, in particular beta-D-oxy-LNA.
- [0142] It will be recognized that when referring to a nucleotide sequence motif or nucleotide sequence, which consists of only nucleotides, the oligomers of the invention which are defined by that sequence, can comprise a corresponding nucleotide analog in place of one or more of the nucleotides present in the sequence, such as LNA units or other nucleotide analogs, including cEt, cMOE, α -LNA, β -LNA, ENA, amino-LNA, oxy-

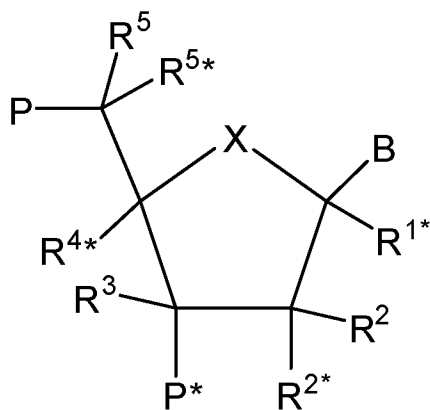
LNA, thio-LNA, which raise the duplex stability/ T_m of the oligomer/target duplex (*i.e.* affinity enhancing nucleotide analogs).

- [0143] In some embodiments, any mismatches between the nucleotide sequence of the oligomer and the target sequence are found in regions outside the affinity enhancing nucleotide analogs, such as region B as referred to herein, and/or region D as referred to herein, and/or at the site of non-modified such as DNA nucleotides in the oligonucleotide, and/or in regions which are 5' or 3' to the contiguous nucleotide sequence.
- [0144] Examples of such modification of the nucleotide include modifying the sugar moiety to provide a 2'-substituent group or to produce a bridged (locked nucleic acid) structure which enhances binding affinity and can also provide increased nuclease resistance.
- [0145] In one embodiment, a nucleotide analog is oxy-LNA (such as beta-D-oxy-LNA, and alpha-L-oxy-LNA), and/or amino-LNA (such as beta-D-amino-LNA and alpha-L-amino-LNA) and/or thio-LNA (such as beta-D-thio-LNA and alpha-L-thio-LNA) and/or ENA (such as beta-D-ENA and alpha-L-ENA). In a particular embodiment, a nucleotide analog is beta-D-oxy-LNA.
- [0146] In some embodiments, the oligomer according to the invention comprises at least one Locked Nucleic Acid (LNA) unit, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 LNA units, such as from 3–7 or 4–8 LNA units, or 3, 4, 5, 6, 7, or 8 LNA units. In some embodiments, all the nucleotide analogs are LNA. In some embodiments, the oligomer can comprise both beta-D-oxy-LNA, and one or more of the following LNA units: thio-LNA, amino-LNA, oxy-LNA, and/or ENA in either the beta-D or alpha-L configurations or combinations thereof. In some embodiments all LNA cytosine units are 5'-methylcytosine. In some embodiments of the invention, the oligomer can comprise both LNA and DNA units. In certain embodiments, the combined total of LNA and DNA units is 10–50, 10–30, such as 10–25, *e.g.*, 10–22, such as 10–21. In some embodiments of the invention, the nucleotide sequence of the oligomer, such as the contiguous nucleotide sequence consists of at least one LNA and the remaining nucleotide units are DNA units. In some embodiments the oligomer comprises only LNA nucleotide analogs and naturally occurring nucleotides (such as RNA or DNA, *e.g.*, DNA nucleotides), optionally with modified internucleotide linkages such as phosphorothioate.

- [0147] The term "nucleobase" refers to the base moiety of a nucleotide and covers both naturally occurring as well as non-naturally occurring variants. Thus, "nucleobase" covers not only the known purine and pyrimidine heterocycles but also heterocyclic analogs and tautomeres thereof.
- [0148] Examples of nucleobases include, but are not limited to adenine, guanine, cytosine, thymidine, uracil, xanthine, hypoxanthine, 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.
- [0149] In some embodiments, at least one of the nucleobases present in the oligomer is a modified nucleobase selected from the group consisting of 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.
- [0150] In certain embodiments, the present invention includes oligomers comprising nucleotide analogs. In some embodiments, the nucleotide analog comprises a modified nucleobase such as 5-methylcytosine. In other embodiments, the nucleotide analog comprise a modified nucleobases such as 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine. In certain embodiments, the oligomers have a chemical structure as disclosed in Figure 1 or Figure 6B.

II.D. LNA

- [0151] The term "LNA" refers to a bicyclic nucleoside analog, known as "Locked Nucleic Acid". It can refer to an LNA monomer, or, when used in the context of an "LNA oligonucleotide," LNA refers to an oligonucleotide containing one or more such bicyclic nucleotide analogs. LNA nucleotides are characterized by the presence of a linker group (such as a bridge) between C2' and C4' of the ribose sugar ring – for example as shown as the biradical R^{4*} - R^{2*} as described below.
- [0152] LNAs may have the structure of the general formula V:



(V)

wherein for all chiral centers, asymmetric groups can be found in either R or S orientation;

wherein X is selected from -O-, -S-, -N(RN*)-, -C(R6R6*)-, such as, in some embodiments -O-;

B is selected from hydrogen, optionally substituted C1-4-alkoxy, optionally substituted C1-4-alkyl, optionally substituted C1-4-acyloxy, nucleobases including naturally occurring and nucleobase analogs, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; in some embodiments, B is a nucleobase or nucleobase analog;

P designates an internucleotide linkage to an adjacent monomer, or a 5'-terminal group, such internucleotide linkage or 5'-terminal group optionally including the substituent R5 or equally applicable the substituent R5*;

P* designates an internucleotide linkage to an adjacent monomer, or a 3'-terminal group;

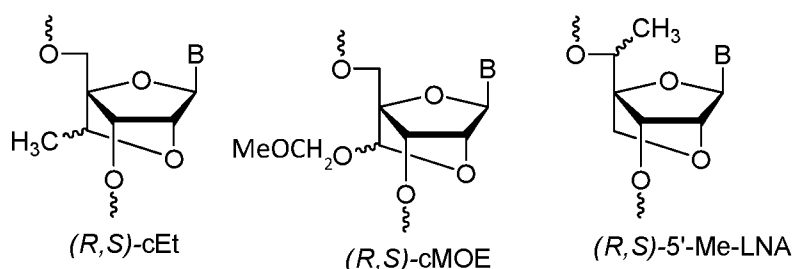
R4* and R2* together designate a bivalent linker group consisting of 1 - 4 groups/atoms selected from -C(RaRb)-, -C(Ra)=C(Rb)-, -C(Ra)=N-, -O-, -Si(Ra)2-, -S-, -SO2-, -N(Ra)-, and >C=Z, wherein Z is selected from -O-, -S-, and -N(Ra)-, and Ra and Rb each is independently selected from hydrogen, optionally substituted C1-12-alkyl, optionally substituted C2-12-alkenyl, optionally substituted C2-12-alkynyl, hydroxy, optionally substituted C1-12-alkoxy, C2-12-alkoxyalkyl, C2-12-alkenyloxy, carboxy, C1-12-alkoxycarbonyl, C1-12-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C1-6-alkyl)amino, carbamoyl, mono- and di(C1-6-alkyl)-amino-carbonyl, amino-C1-6-alkyl-aminocarbonyl, mono- and di(C1-6-alkyl)amino-C1-6-alkyl-aminocarbonyl, C1-6-alkyl-carbonylamino, carbamido, C1-6-alkanoyloxy, sulphono, C1-

6-alkylsulphonyloxy, nitro, azido, sulphanyl, C1-6-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl can be optionally substituted and where two geminal substituents Ra and Rb together can designate optionally substituted methylene (=CH₂), wherein for all chiral centers, asymmetric groups can be found in either R or S orientation, and; each of the substituents R1*, R2, R3, R5, R5*, R6 and R6*, which are present is independently selected from hydrogen, optionally substituted C1-12-alkyl, optionally substituted C2-12-alkenyl, optionally substituted C2-12-alkynyl, hydroxy, C1-12-alkoxy, C2-12-alkoxyalkyl, C2-12-alkenyloxy, carboxy, C1-12-alkoxycarbonyl, C1-12-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C1-6-alkyl)amino, carbamoyl, mono- and di(C1-6-alkyl)-amino-carbonyl, amino-C1-6-alkyl-aminocarbonyl, mono- and di(C1-6-alkyl)amino-C1-6-alkyl-aminocarbonyl, C1-6-alkyl-carbonylamino, carbamido, C1-6-alkanoyloxy, sulphono, C1-6-alkylsulphonyloxy, nitro, azido, sulphanyl, C1-6-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl can be optionally substituted, and where two geminal substituents together can designate oxo, thioxo, imino, or optionally substituted methylene; ; wherein RN is selected from hydrogen and C1-4-alkyl, and where two adjacent (non-geminal) substituents can designate an additional bond resulting in a double bond; and RN*, when present and not involved in a biradical, is selected from hydrogen and C1-4-alkyl; and basic salts and acid addition salts thereof. For all chiral centers, asymmetric groups can be found in either R or S orientation.

[0153] In some embodiments, R4* and R2* together designate a biradical consisting of a groups selected from the group consisting of C(RaRb)-C(RaRb)-, C(RaRb)-O-, C(RaRb)-NRa-, C(RaRb)-S-, and C(RaRb)-C(RaRb)-O-, wherein each Ra and Rb can optionally be independently selected. In some embodiments, Ra and Rb can be, optionally independently selected from the group consisting of hydrogen and C1-6alkyl, such as methyl, such as hydrogen.

[0154] In some embodiments, R^{4*} and R^{2*} together designate the biradical -O-CH(CH₂OCH₃)- (2'O-methoxyethyl bicyclic nucleic acid - Seth et al., 2010, J. Org. Chem) – in either the R- or S- configuration.

- [0155] In some embodiments, R^{4*} and R^{2*} together designate the biradical $-O-CH(CH_2CH_3)-$ (2'O-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem). – in either the *R*- or *S*- configuration.
- [0156] In some embodiments, R^{4*} and R^{2*} together designate the biradical $-O-CH(CH_3)-$. – in either the *R*- or *S*- configuration. In some embodiments, R^{4*} and R^{2*} together designate the biradical $-O-CH_2-O-CH_2-$ - (Seth at al., 2010, J. Org. Chem).
- [0157] In some embodiments, R^{4*} and R^{2*} together designate the biradical $-O-NR-CH_3-$ - (Seth at al., 2010, J. Org. Chem).
- [0158] In some embodiments, the LNA units have a structure selected from the following group:



- [0159] In some embodiments, the oligomer of the invention does not comprise (*R*, *S*)cET, cMOE or 5'Me-LNA units. In some embodiments, the oligomer of the invention does not comprise (*S*)cET LNA units.
- [0160] In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation.
- [0161] In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are hydrogen.
- [0162] In some embodiments, R^{1*} , R^2 , R^3 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation.
- [0163] In some embodiments, R^{1*} , R^2 , R^3 are hydrogen.

[0164] In some embodiments, R^5 and R^{5*} are each independently selected from the group consisting of H, $-\text{CH}_3$, $-\text{CH}_2\text{-CH}_3$, $-\text{CH}_2\text{-O-CH}_3$, and $-\text{CH=CH}_2$. Suitably in some embodiments, either R^5 or R^{5*} are hydrogen, whereas the other group (R^5 or R^{5*} respectively) is selected from the group consisting of C_{1-5} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl or substituted acyl ($-\text{C(=O)-}$); wherein each substituted group is mono or poly substituted with substituent groups independently selected from halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, OJ_1 , SJ_1 , NJ_1J_2 , N_3 , COOJ_1 , CN , $\text{O-C(=O)NJ}_1\text{J}_2$, $\text{N(H)C(=NH)NJ}_1\text{J}_2$ or N(H)C(X)N(H)J_2 wherein X is O or S; and each J_1 and J_2 is, independently, H, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} aminoalkyl, substituted C_{1-6} aminoalkyl or a protecting group. In some embodiments either R^5 or R^{5*} is substituted C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is substituted methylene wherein preferred substituent groups include one or more groups independently selected from F, NJ_1J_2 , N_3 , CN , OJ_1 , SJ_1 , $\text{O-C(=O)NJ}_1\text{J}_2$, $\text{N(H)C(=NH)NJ}_1\text{J}_2$ or N(H)C(O)N(H)J_2 . In some embodiments each J_1 and J_2 is, independently H or C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is methyl, ethyl or methoxymethyl. In some embodiments either R^5 or R^{5*} is methyl. In a further embodiment either R^5 or R^{5*} is ethylenyl. In some embodiments either R^5 or R^{5*} is substituted acyl. In some embodiments either R^5 or R^{5*} is $\text{C(=O)NJ}_1\text{J}_2$. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation. Such 5' modified bicyclic nucleotides are disclosed in WO 2007/134181, which is hereby incorporated by reference in its entirety.

[0165] In some embodiments B is a nucleobase, including nucleobase analogs and naturally occurring nucleobases, such as a purine or pyrimidine, or a substituted purine or substituted pyrimidine, such as a nucleobase referred to herein, such as a nucleobase selected from the group consisting of adenine, cytosine, thymine, adenine, uracil, and/or a modified or substituted nucleobase, such as 5-thiazolo-uracil, 2-thio-uracil, 5-propynyl-uracil, 2-thio-thymine, 5-methyl cytosine, 5-thiazolo-cytosine, 5-propynyl-cytosine, and 2,6-diaminopurine.

[0166] In some embodiments, R^{4*} and R^{2*} together designate a biradical selected from - $\text{C(R}^a\text{R}^b\text{)-O-}$, - $\text{C(R}^a\text{R}^b\text{)-C(R}^c\text{R}^d\text{)-O-}$, - $\text{C(R}^a\text{R}^b\text{)-C(R}^c\text{R}^d\text{)-C(R}^e\text{R}^f\text{)-O-}$, - $\text{C(R}^a\text{R}^b\text{)-O-C(R}^c\text{R}^d\text{)-}$, - $\text{C(R}^a\text{R}^b\text{)-O-C(R}^c\text{R}^d\text{)-O-}$, - $\text{C(R}^a\text{R}^b\text{)-C(R}^c\text{R}^d\text{)-}$, - $\text{C(R}^a\text{R}^b\text{)-C(R}^c\text{R}^d\text{)-C(R}^e\text{R}^f\text{)-}$, -

$C(R^a)=C(R^b)-C(R^cR^d)-$, $-C(R^aR^b)-N(R^c)-$, $-C(R^aR^b)-C(R^cR^d)-N(R^e)-$, $-C(R^aR^b)-N(R^c)-O-$, and $-C(R^aR^b)-S-$, $-C(R^aR^b)-C(R^cR^d)-S-$, wherein R^a , R^b , R^c , R^d , R^e , and R^f each is independently selected from hydrogen, optionally substituted C_{1-12} -alkyl, optionally substituted C_{2-12} -alkenyl, optionally substituted C_{2-12} -alkynyl, hydroxy, C_{1-12} -alkoxy, C_{2-12} -alkoxyalkyl, C_{2-12} -alkenyloxy, carboxy, C_{1-12} -alkoxycarbonyl, C_{1-12} -alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6} -alkyl)amino, carbamoyl, mono- and di(C_{1-6} -alkyl)-amino-carbonyl, amino- C_{1-6} -alkyl-aminocarbonyl, mono- and di(C_{1-6} -alkyl)amino- C_{1-6} -alkyl-aminocarbonyl, C_{1-6} -alkyl-carbonylamino, carbamido, C_{1-6} -alkanoyloxy, sulphono, C_{1-6} -alkylsulphonyloxy, nitro, azido, sulphonyl, C_{1-6} -alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl can be optionally substituted and where two geminal substituents R^a and R^b together can designate optionally substituted methylene ($=CH_2$). For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation.

[0167] In a further embodiment R^{4*} and R^{2*} together designate a biradical (bivalent group) selected from $-CH_2-O-$, $-CH_2-S-$, $-CH_2-NH-$, $-CH_2-N(CH_3)-$, $-CH_2-CH_2-O-$, $-CH_2-CH(CH_3)-$, $-CH_2-CH_2-S-$, $-CH_2-CH_2-NH-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-O-$, $-CH_2-CH_2-CH(CH_3)-$, $-CH=CH-CH_2-$, $-CH_2-O-CH_2-O-$, $-CH_2-NH-O-$, $-CH_2-N(CH_3)-O-$, $-CH_2-O-CH_2-$, $-CH(CH_3)-O-$, and $-CH(CH_2-O-CH_3)-O-$, and/or, $-CH_2-CH_2-$, and $-CH=CH-$. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation.

[0168] In some embodiments, R^{4*} and R^{2*} together designate the biradical $C(R^aR^b)-N(R^c)-O-$, wherein R^a and R^b are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen, and; wherein R^c is selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

[0169] In some embodiments, R^{4*} and R^{2*} together designate the biradical $C(R^aR^b)-O-C(R^cR^d)-O-$, wherein R^a , R^b , R^c , and R^d are independently selected from the group

consisting of hydrogen, halogen, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, C₂₋₆ alkenyl, substituted C₂₋₆ alkenyl, C₂₋₆ alkynyl or substituted C₂₋₆ alkynyl, C₁₋₆ alkoxy, substituted C₁₋₆ alkoxy, acyl, substituted acyl, C₁₋₆ aminoalkyl or substituted C₁₋₆ aminoalkyl, such as hydrogen.

[0170] In some embodiments, R^{4*} and R^{2*} form the biradical –CH(Z)-O-, wherein Z is selected from the group consisting of C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, substituted C₁₋₆ alkyl, substituted C₂₋₆ alkenyl, substituted C₂₋₆ alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio; and wherein each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJ₁, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ³C(=X)NJ₁J₂ and CN, wherein each J₁, J₂ and J₃ is, independently, H or C₁₋₆ alkyl, and X is O, S or NJ₁. In some embodiments Z is C₁₋₆ alkyl or substituted C₁₋₆ alkyl. In some embodiments Z is methyl. In some embodiments Z is substituted C₁₋₆ alkyl. In some embodiments the substituent group is C₁₋₆ alkoxy. In some embodiments Z is CH₃OCH₂-. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in US 7,399,845 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*}, R², R³, R⁵, R^{5*} are hydrogen. In some embodiments, R^{1*}, R², R^{3*} are hydrogen, and one or both of R⁵, R^{5*} can be other than hydrogen as referred to above and in WO 2007/134181, which is incorporated by reference herein in its entirety.

[0171] In some embodiments, R^{4*} and R^{2*} together designate a biradical which comprise a substituted amino group in the bridge such as consist of or comprise the biradical –CH₂-N(R^c)-, wherein R^c is C₁₋₁₂ alkyloxy. In some embodiments R^{4*} and R^{2*} together designate a biradical –Cq₃q₄-NOR -, wherein q₃ and q₄ are independently selected from the group consisting of hydrogen, halogen, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, C₂₋₆ alkenyl, substituted C₂₋₆ alkenyl, C₂₋₆ alkynyl or substituted C₂₋₆ alkynyl, C₁₋₆ alkoxy, substituted C₁₋₆ alkoxy, acyl, substituted acyl, C₁₋₆ aminoalkyl or substituted C₁₋₆ aminoalkyl; wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJ₁, SJ₁, NJ₁J₂, COOJ₁, CN, O-C(=O)NJ₁J₂, N(H)C(=NH)N J₁J₂ or N(H)C(=X=N(H)J₂ wherein X is O or S; and each of J₁ and J₂ is, independently, H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ aminoalkyl or a protecting group. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/150729 which is hereby

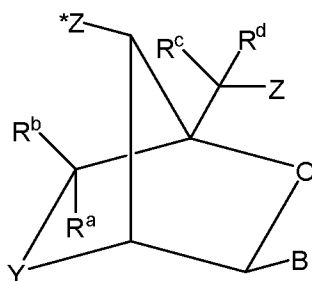
incorporated by reference in its entirety. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are hydrogen. In some embodiments, R^{1*} , R^2 , R^3 are hydrogen and one or both of R^5 , R^{5*} can be other than hydrogen as referred to above and in WO 2007/134181. In some embodiments R^{4*} and R^{2*} together designate a biradical (bivalent group) $C(R^a R^b)-O-$, wherein R^a and R^b are each independently halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_1 , SJ_1 , SOJ_1 , SO_2J_1 , NJ_1J_2 , N_3 , CN , $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O-C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; or R^a and R^b together are $=C(q_3)(q_4)$; q_3 and q_4 are each, independently, H, halogen, C_1-C_{12} alkyl or substituted C_1-C_{12} alkyl; each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, OJ_1 , SJ_1 , NJ_1J_2 , N_3 , CN , $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O-C(=O)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; and; each J_1 and J_2 is, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, C_1-C_6 aminoalkyl, substituted C_1-C_6 aminoalkyl or a protecting group. Such compounds are disclosed in WO2009006478A, hereby incorporated in its entirety by reference.

[0172] In some embodiments, R^{4*} and R^{2*} form the biradical - Q -, wherein Q is $C(q_1)(q_2)C(q_3)(q_4)$, $C(q_1)=C(q_3)$, $C[=C(q_1)(q_2)]-C(q_3)(q_4)$ or $C(q_1)(q_2)-C[=C(q_3)(q_4)]$; q_1 , q_2 , q_3 , q_4 are each independently, H, halogen, C_{1-12} alkyl, substituted C_{1-12} alkyl, C_{2-12} alkenyl, substituted C_{1-12} alkoxy, OJ_1 , SJ_1 , SOJ_1 , SO_2J_1 , NJ_1J_2 , N_3 , CN , $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $-C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; each J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group; and, optionally wherein when Q is $C(q_1)(q_2)(q_3)(q_4)$ and one of q_3 or q_4 is CH_3 then at least one of the other of q_3 or q_4 or one of q_1 and q_2 is other than H. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are hydrogen. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed

in WO2008/154401 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are hydrogen. In some embodiments, R^{1*} , R^2 , R^3 are hydrogen and one or both of R^5 , R^{5*} can be other than hydrogen as referred to above and in WO 2007/134181 or WO2009/067647 (alpha-L-bicyclic nucleic acids analogs).

[0173] Further bicyclic nucleoside analogs and their use in antisense oligonucleotides are disclosed in WO2011/115818, WO2011/085102, WO2011/017521, WO09/100320, WO10/036698, WO09/124295 & WO09/006478, each of which are incorporated by reference herein in their entireties. Such nucleoside analogs can in some aspects be useful in the compounds of present invention.

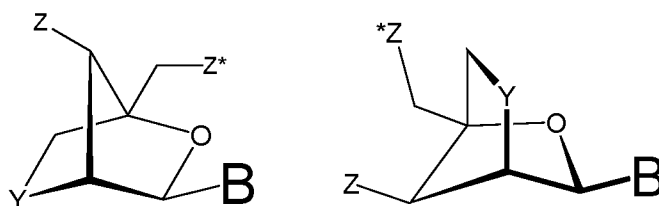
[0174] In some embodiments the LNA used in the oligonucleotide compounds of the invention has the structure of the general formula VI:



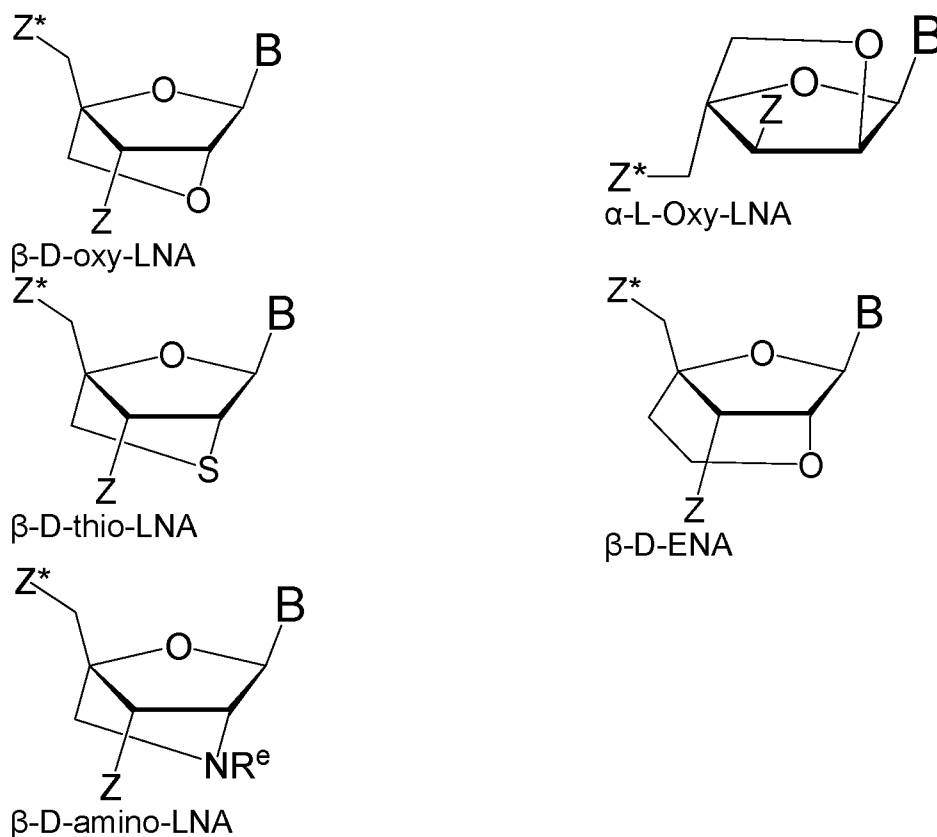
(VI)

wherein Y is selected from the group consisting of -O-, -CH₂O-, -S-, -NH-, N(R^c) and/or -CH₂-; Z and Z* are independently selected among an internucleotide linkage, R^H, a terminal group or a protecting group; B constitutes a natural or non-natural nucleotide base moiety (nucleobase), and R^H is selected from hydrogen and C_{1-4} -alkyl; R^a , R^b , R^c , R^d and R^e are, optionally independently, selected from the group consisting of hydrogen, optionally substituted C_{1-12} -alkyl, optionally substituted C_{2-12} -alkenyl, optionally substituted C_{2-12} -alkynyl, hydroxy, C_{1-12} -alkoxy, C_{2-12} -alkoxyalkyl, C_{2-12} -alkenyloxy, carboxy, C_{1-12} -alkoxycarbonyl, C_{1-12} -alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6} -alkyl)amino, carbamoyl, mono- and di(C_{1-6} -alkyl)-amino-carbonyl, amino- C_{1-6} -alkyl-aminocarbonyl, mono- and di(C_{1-6} -alkyl)amino- C_{1-6} -alkyl-aminocarbonyl, C_{1-6} -alkyl-carbonylamino, carbamido, C_{1-6} -

alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphonyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl can be optionally substituted and where two geminal substituents R^a and R^b together can designate optionally substituted methylene (=CH₂); and R^H is selected from hydrogen and C₁₋₄-alkyl. In some embodiments R^a, R^b, R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen and C₁₋₆ alkyl, such as methyl. For all chiral centers, asymmetric groups can be found in either *R* or *S* orientation, for example, two exemplary stereochemical isomers include the beta-D and alpha-L isoforms, which can be illustrated as follows:



[0175] Specific exemplary LNA units are shown below:



- [0176] In some embodiments, the oligomer of the invention comprises beta-D-oxy-LNA units, and if present, other LNA units selected from the group consisting of alpha-L-LNA, beta-D-thio-LNA, beta-D-amino LNA and beta-D-ENA-LNA. In some embodiments, all LNA units present in the oligomer of the invention are beta-D-oxy-LNA units
- [0177] In other embodiments, the oligomers of the invention comprise nucleotides with modified sugar moieties as described in Figures 1, 2, 3, 4, 6A, 6B, 11A and 11B.

II.E. RNase recruitment

- [0178] It is recognized that an oligomeric compound can function via non RNase mediated degradation of target mRNA, such as by steric hindrance of translation, or other methods, however, in one aspect, the oligomers of the invention are capable of recruiting an endoribonuclease (RNase), such as RNaseH.
- [0179] In one aspect, the oligomer, or contiguous nucleotide sequence, comprises a region of at least 7 consecutive nucleotide units, such as at least 8 or at least 9 consecutive nucleotide units (residues), in certain embodiments including 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 consecutive nucleotides, which, when formed in a duplex with the complementary target RNA is capable of recruiting RNase. The contiguous sequence which is capable of recruiting RNase can be region B as referred to in the context of a gapmer as described herein. In some embodiments the size of the contiguous sequence which is capable of recruiting RNase, such as region B, can be higher, such as 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotide units.
- [0180] US Patent No. 6,617,442, which is incorporated by reference herein in its entirety, provides *in vitro* methods for determining RNaseH activity, which can be used to determine the ability to recruit RNaseH. Therefore, in one embodiment, an oligomer of the invention is capable of recruiting RNaseH. In another embodiment, the invention includes a method of identifying an oligomer which is capable of utilizing RNaseH mechanism, *e.g.*, recruiting RNaseH.
- [0181] Oligomers can be screened to identify those which are effective in recruiting RNaseH. The ability of oligomers to recruit RNaseH can be determined by measuring the binding of the oligomers to RNaseH. The methods of determining binding of the oligomers to RNaseH are well known in the art. For example, the oligomers can be radiolabeled and binding of the oligomers to RNaseH can be detected by

autoradiography. In some embodiments, fusion proteins of RNaseH with glutathione-S-transferase or small peptide tags can be prepared and immobilized to a solid phase such as beads. Labeled or unlabeled oligomers to be screened for binding to this enzyme can then be incubated with the solid phase. Oligomers which bind to the enzyme immobilized to the solid phase can then be identified either by detection of bound label or by eluting specifically the bound oligomers from the solid phase. Another method involves screening of oligomer libraries for binding partners. Recombinant tagged or labeled RNaseH is used to select oligomers from the library which interact with the enzyme. Sequencing of the oligomers leads to identification of those oligomers which will be more effective as antisense molecules.

[0182] An oligomer is deemed capable of recruiting RNaseH if, when provided with the complementary RNA target, it has an initial rate, as measured in pmol/l/min, of at least 1 %, such as at least 5%, such as at least 10% or ,more than 20% of the of the initial rate determined using DNA only oligonucleotide, having the same base sequence but containing only DNA monomers, with no 2' substitutions, with phosphorothioate linkage groups between all monomers in the oligonucleotide,, using the methodology provided by Example 91 - 95 of US Patent No. 6,617,442.

[0183] In some embodiments, an oligomer is deemed essentially incapable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is less than 1%, such as less than 5%,such as less than 10% or less than 20% of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of US Patent No. 6,617,442.

[0184] In other embodiments, an oligomer is deemed capable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is at least 20%, such as at least 40 %, such as at least 60 %, such as at least 80 % of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of US Patent No. 6,617,442.

[0185] Typically the region of the oligomer which forms the consecutive nucleotide units which, when formed in a duplex with the complementary target RNA is capable of recruiting RNase consists of nucleotide units which form a DNA/RNA like duplex with the RNA target – and include both DNA units and LNA units which are in the alpha-L configuration, particularly preferred being alpha-L-oxy LNA.

[0186] In some embodiments, the monomers which are capable of recruiting RNase are selected from the group consisting of DNA monomers, alpha-L-LNA monomers, C4' alkylated DNA monomers (see PCT/EP2009/050349 and Vester *et al.*, Bioorg. Med. Chem. Lett. 18 (2008) 2296–2300, hereby incorporated by reference in its entirety), and UNA (unlinked nucleic acid) nucleotides (see Fluiter *et al.*, Mol. Biosyst., 2009, 10, 1039, hereby incorporated by reference). UNA is unlocked nucleic acid, typically where the C2–C3 C-C bond of the ribose has been removed, forming an unlocked "sugar" residue.

II.F. Oligomer Design

[0187] The oligomer of the invention can comprise a nucleotide sequence which comprises both nucleotides and nucleotide analogs, and can be in the form of a gapmer, blockmer, mixmer, headmer, tailmer, or totalmer. Examples of configurations of a gapmer, blockmer, mixmer, headmer, tailmer, or totalmer that can be used with the oligomer of the invention are described in U.S. Patent Appl. Publ. No. 2012/0322851, which is incorporated by reference herein in its entirety.

[0188] Non-limiting examples of the oligomers are disclosed in the Figures herein to show representative designs and sequences, but the oligomer designs are not limited with the particular sequences disclosed herein.

[0189] A gapmer oligomer is an oligomer which comprises a contiguous stretch of nucleotides which is capable of recruiting an RNase, such as RNaseH, such as a region of at least 5 DNA nucleotides, at least 6 DNA nucleotides, or at least 7 DNA nucleotides, which is flanked both 5' and 3' by regions of affinity enhancing (e.g., 1-6) nucleotide analogs 5' and 3' to the contiguous stretch of nucleotides which is capable of recruiting RNase.

[0190] The term "gapmer" as used herein can include a traditional gapmer (e.g., oligomer with a design of G-H-I, wherein region H comprises DNAs only, and regions G and I comprise LNAs only) or a gapmer with an alternating flank at either wing position. The

term "alternating flank" as referred to herein means a contiguous sequence of at least three nucleosides comprising LD₁₋₆L in any portion of the sequence wherein L is LNA and D is DNA (e.g., LDL, LDDL, LDDDL, LDDDDL, LDDDDDL, or LDDDDDDL).

- [0191] In some embodiments, in addition to enhancing affinity of the oligomer for the target region, some nucleoside analogs also mediate RNase (*e.g.*, RNaseH) binding and cleavage. Since α -L-LNA monomers recruit RNaseH activity to a certain extent, in some embodiments, gap regions (*e.g.*, region B as referred to herein) of oligomers containing α -L-LNA monomers consist of fewer monomers recognizable and cleavable by the RNaseH, and more flexibility in the mixmer construction is introduced.
- [0192] The oligomer of the invention comprises region A, region B, and region C (A-B-C), wherein region B comprises at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 consecutive nucleoside units and is flanked at 5' by region A of 1-8 contiguous nucleoside units and at 3' by region C of 1-8 contiguous nucleoside units, wherein region B, when formed in a duplex with a complementary RNA, is capable of recruiting RNaseH, and wherein at least one of region A and region C comprises at least one terminal LNA nucleotide, a DNA nucleoside, and a further LNA nucleoside adjacent to region B.
- [0193] In some embodiments, region A comprises a 5' LNA nucleoside unit and a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit, and region C comprises at least two 3' LNA nucleoside units.
- [0194] In some embodiments, region C comprises a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units, and region A comprises at least one 5' LNA nucleoside unit.
- [0195] In some embodiments, region A comprises a 5' LNA nucleoside unit and a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit; and region C comprises a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units.
- [0196] In certain embodiments, an oligomer design can provide an enhanced property, *e.g.*, reducing non-specific binding while retaining affinity to the target nucleic acid. For

example, while not being bound by any theory, the present disclosure provides an oligomer that retains affinity for a target nucleic acid that is comparable to a corresponding reference gapmer, but exhibits reduced non-specific binding to non-target nucleic acids compared to the corresponding reference gapmer. In other embodiments, the oligomers of the invention further comprise one or more enhanced properties, e.g., stability, less toxicity, etc.

[0197] In some embodiments, the oligomers of the invention have reduced toxicity in vitro and/or in vivo compared to a corresponding reference oligomer that does not have the design described herein (e.g., a gapmer with one or two alternating flanks). In certain embodiments, the alternating flank gapmer exhibits less off target binding compared to a corresponding reference oligomer that does not have the design described herein. In some embodiments, the corresponding reference oligomer has the same sequence as the oligomer of the present invention, but has a gapmer design of G-H-I, wherein region H is identical to region B, region G has the same sequence as region A except that the nucleosides in region G are all LNAs, and region I has the same sequence as region C except that the nucleosides in region I are all LNAs.

[0198] In some embodiments, either region A or region C comprises 1, 2, or 3 DNA nucleoside units. In some embodiments, region A and region C each comprise 1, 2, or 3 DNA nucleoside units.

[0199] In some embodiments, region B comprises at least five consecutive DNA nucleoside units. In some embodiments, region B comprises 6, 7, 8, 9, 10, 11, 12, 13 or 14 consecutive DNA nucleoside units. In some embodiments, region B is 8, 9, 10, 11, or 12 nucleotides in length.

[0200] In some embodiments, region A comprises two 5' terminal LNA nucleoside units. In some embodiments, region A has formula 5' [LNA]₁₋₃[DNA]₁₋₃[LNA]₁₋₃, or 5' [LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂[DNA]₁₋₂[LNA]₁₋₂.

[0201] The term [LNA]_{x-y} as used herein means the number of LNA units in a contiguous sequence. The minimum number of LNA units that can be present in the sequence is x and the maximum number of LNA units that can be present in the sequence is y.

[0202] When region A of 1-8 contiguous nucleoside units has formula 5' [LNA]₁₋₃[DNA]₁₋₃[LNA]₁₋₃, the total number of [LNA][DNA][LNA] may not be higher than 8 contiguous

nucleoside units (e.g., 3-3-2, 3-2-3, or 2-3-3) while one or two of the first [LNA], [DNA] and the second [LNA] can be as high as 3.

[0203] In some embodiments, region C has formula $[LNA]_{1-3}[DNA]_{1-3}[LNA]_{2-3}3'$, or $[LNA]_{1-2}[DNA]_{1-2}[LNA]_{1-2}[DNA]_{1-2}[LNA]_{2-3}3'$.

[0204] In some embodiments, region A has formula $5'[LNA]_{1-3}[DNA]_{1-3}[LNA]_{1-3}$ or $5'[LNA]_{1-2}[DNA]_{1-2}[LNA]_{1-2}[DNA]_{1-2}[LNA]_{1-2}$, and region C comprises 2, 3, 4 or 5 consecutive LNA nucleoside units.

[0205] In some embodiments, region C has formula $[LNA]_{1-3}[DNA]_{1-3}[LNA]_{2-3}3'$ or $[LNA]_{1-2}[DNA]_{1-2}[LNA]_{1-2}[DNA]_{1-2}[LNA]_{2-3}3'$, and region A comprises 1, 2, 3, 4 or 5 consecutive LNA nucleoside units.

[0206] In some embodiments, region A has a sequence of LNA and DNA nucleosides, $5' - 3'$ selected from the group consisting of L, LL, LDL, LLL, LLDL, LDLL, LDDL, LLLL, LLLL, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDDL, LLLLL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LDDDLL, LDDLLL, LDLLDL, LDLDLL, LDDDLL, LLDDDL, and LDLDLD, wherein L represents a LNA nucleoside, and D represents a DNA nucleoside.

[0207] In some embodiments, region C has a sequence of LNA and DNA nucleosides, $5' - 3'$ selected from the group consisting of LL, LLL, LLLL, LDLL, LLLL, LLDLL, LDLLL, LDDL, LDDLLL, LLDDL, LDLDLL, LDDDLL, LDLDLL, LDDLDLL, LDDDLL, and LLDLDLL.

[0208] In some embodiments, region A has a sequence of LNA and DNA nucleosides, $5' - 3'$ selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDDL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LLDDL, LDDLLL, LDLLDL, LDLDLL, LDDDLL, LLDDDL, and LDLDLD, and region C has a sequence of LNA and DNA nucleosides, $5' - 3'$ selected from the group consisting of LDLL, LLLL, LLDLL, LDLLL, LDDL, LDDLLL, LLDDL, LDLDLL, LDDDLL, LDLDLL, LDDLDLL, LDDDLL, and LLDLDLL.

[0209] In some embodiments, the contiguous nucleotides comprises an alternating sequence of LNA and DNA nucleoside units, $5' - 3'$, selected from the group consisting of: 2-3-2-8-2, 1-1-2-1-1-9-2, 3-10-1-1-2, 3-9-1-2-2, 3-8-1-3-2, 3-8-1-1-1-1-2, 3-1-1-9-3, 3-1-1-8-1-1-2, 4-9-1-1-2, 4-8-1-2-2, 3-3-1-8-2, 3-2-1-9-2, 3-2-2-8-2, 3-2-2-7-3, 5-7-1-2-2,

1-1-3-10-2, 1-1-3-7-1-2-2, 1-1-4-9-2, 2-1-3-9-2, 3-1-1-10-2, 3-1-1-7-1-2-2, 3-1-2-9-2, 4-7-1-3-2, 5-9-1-1-2, 4-10-1-1-2, 3-11-1-1-2, 2-1-1-10-1-1-2, 1-1-3-9-1-1-2, 3-10-1-2-2, 3-9-1-3-2, 3-8-1-1-1-2-2, 4-9-1-2-2, 4-9-1-1-3, 4-8-1-3-2, 4-8-1-2-3, 4-8-1-1-1-1-2, 4-7-1-2-1-1-2, 4-7-1-1-1-2-2, 2-1-2-11-2, 2-1-3-8-1-1-2, 3-1-1-11-2, 3-1-1-9-1-1-2, 3-1-1-8-1-2-2, 3-1-1-7-1-1-1-1-2, 4-9-2-1-2, 4-7-1-3-3, 5-9-1-1-3, 5-9-1-2-2, 4-10-2-1-2, 4-10-1-1-3, 4-10-1-2-2, 3-11-2-1-2, 3-11-1-1-3, 5-9-2-1-2, 3-11-1-2-2, 2-1-2-9-1-2-2, 3-1-1-10-1-1-2, 3-1-1-9-1-2-2, 4-9-1-1-1-1-2, 4-8-2-1-1-1-2, 1-1-3-10-2-1-2, 2-1-2-10-2-1-2, 2-1-1-12-4, 2-2-1-11-4, 3-1-1-11-4, 2-1-1-13-3, 2-1-2-11-4, 2-2-1-12-3, 3-11-1-2-3, 3-1-1-12-3, 2-1-2-12-3, 4-11-2-1-2, 4-10-2-2-2, 3-2-1-9-1-1-3, 2-2-1-1-1-9-4, 2-2-2-9-1-1-3, 3-1-1-9-1-1-1-1-2, 2-1-2-9-1-2-3, 3-1-1-10-1-1-3, 2-1-1-2-1-9-4, 4-9-1-1-1-2-2, 3-1-1-9-1-2-3, 2-1-1-1-1-10-4, 2-1-2-10-1-1-3, 2-1-1-1-1-9-2-1-2, 2-2-2-9-2-1-2, 4-9-1-2-1-1-2, 3-2-1-9-2-1-2, 2-1-2-9-2-2-2, 2-1-1-1-1-9-1-1-3, 3-1-1-9-2-2-2, 2-2-2-10-4, 2-1-2-9-1-1-1-1-2, 4-10-1-2-3, 3-2-1-10-4, 3-1-1-10-2-1-2, 4-10-1-1-1-1-2, 4-11-1-1-3, and 2-2-2-10-1-1-2; wherein the first numeral represents the number of LNA units, the next the number of DNA units, and alternating LNA and DNA regions thereafter.

[0210] In some embodiments, the maximum number of contiguous LNA nucleosides in regions A and C is 2 or 3. In some embodiments, the maximum number of contiguous DNA nucleosides in regions A and C is 1 or 2.

[0211] In some embodiments, the LNA nucleoside units present in regions A and C are beta-D LNA units, such as beta-D-oxy LNA nucleoside units or (S)cEt nucleoside LNA units.

[0212] In some embodiments, the oligomer comprises at least one phosphorothioate internucleoside linkage.

[0213] In some embodiments, the internucleoside linkages within region B are phosphorothioate internucleoside linkages.

[0214] In some embodiments, all the internucleoside linkages within regions A, B and C are phosphorothioate internucleoside linkages.

[0215] In one embodiment, the oligomer of the invention is a gapmer. A gapmer oligomer is an oligomer which comprises a contiguous stretch of nucleotides which is capable of recruiting an RNase, such as RNaseH, such as a region of at least 7 DNA nucleotides, referred to herein in as region B (B), wherein region B is flanked both 5' and 3' by regions of affinity enhancing nucleotide analogs, such as from 1–10 nucleotide analogs 5' and 3'

to the contiguous stretch of nucleotides which is capable of recruiting RNase – these regions are referred to as regions A (A) and C (C) respectively.

[0216] In the gapmers of the invention, at least one of region A or C, or both regions A and C, comprise at least one DNA nucleoside.

[0217] In certain embodiments, the gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), A-B-C, wherein: region A (A) (5' region or a first wing sequence) comprises at least one nucleotide analog, such as at least one LNA unit, such as from 1-10 nucleotide analogs, such as LNA units, and; region B (B) comprises at least seven consecutive nucleotides which are capable of recruiting RNase (when formed in a duplex with a complementary RNA molecule, such as the pre-mRNA or mRNA target), such as DNA nucleotides, and; region C (C) (3' region or a second wing sequence) comprises at least one nucleotide analog, such as at least one LNA unit, such as from 1-10 nucleotide analogs, such as LNA units; wherein regions A and C may include at any position in A and C 1-2 insertions of DNA nucleotide regions (e.g., DNA gapmers), in which these DNA insertions may each be 1-3 DNA units long.

[0218] In certain other embodiments, the gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein: region A (A) (5' region) comprises at least one nucleotide analog, such as at least one LNA unit, such as from 1-10 nucleotide analogs, such as LNA units, and; region B (B) comprises at least seven consecutive nucleotides which are capable of recruiting RNase (when formed in a duplex with a complementary RNA molecule, such as the mRNA target), such as DNA nucleotides, and; region C (C) (3' region) comprises at least one nucleotide analog, such as at least one LNA unit, such as from 1-10 nucleotide analogs, such as LNA units, and; region D (D), when present comprises 1, 2 or 3 nucleotide units, such as DNA nucleotides.

[0219] In some embodiments, region A comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide analogs, such as LNA units, such as from 2-5 nucleotide analogs, such as 2-5 LNA units, such as 2-5 nucleotide analogs, such as 3-5 LNA units; and/or region C consists of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide analogs, such as LNA units, such as from 2-5 nucleotide analogs, such as 2-5 LNA units, such as 2-5 nucleotide analogs, such as 3-5 LNA units.

[0220] In some embodiments B comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 consecutive nucleotides which are capable of recruiting RNase, or

from 8-14, or from 7-10, or from 7-9, such as 8, such as 9, such as 10, or such as 14 consecutive nucleotides which are capable of recruiting RNase. In some embodiments region B comprises at least seven DNA nucleotide unit, such as 7-23 DNA units, such as from 7-20 DNA units, such as from 7-14 DNA units, such as from 8-14 DNA units, such as 7, 8, 9, 10, 11, 12, 13, or 14 DNA units.

[0221] In some embodiments region A comprises 3, 4, or 5 nucleotide analogs, such as LNA, region B consists of 7, 8, 9, 10, 11, 12, 13, or 14 DNA units, and region C consists of 3, 4, or 5 nucleotide analogs, such as LNA. Such designs include (A-B-C) 5-10-5, 3-14-3, 3-10-3, 3-10-4, 4-10-3, 3-9-3, 3-9-4, 4-9-3, 3-8-3, 3-8-4, 4-8-3, 3-7-3, 3-7-4, 4-7-3, and can further include region D, which can have one to 3 nucleotide units, such as DNA units.

[0222] In some embodiments, the oligomer of the invention has the formula of 5'-A-B-C-3', wherein

- (i) B is a contiguous sequence of 7 to 23 DNA units;
- (ii) A is a first wing sequence of 1 to 10 nucleotides, wherein the first wing sequence comprises one or more nucleotide analogs and optionally one or more DNA units (e.g., DNA gapmer) and wherein at least one of the nucleotide analogs is located at the 5' end of A; and
- (iii) C is a second wing sequence of 1 to 10 nucleotides, wherein the second wing sequence comprises one or more nucleotide analogs and optionally one or more DNA units (e.g., DNA gapmer) and wherein at least one of the nucleotide analogs is located at the 3' end of C.

[0223] In other embodiments, the oligomer has the formula of 5'-A-B-C-3', wherein B is a contiguous sequence of 7 to 23 DNA units, A is LmDnLoDpLq and C is Lm'Dn'Lo'Dp'Lq' and wherein L is a nucleotide analog; D is a DNA unit; m and q' are 1 to 6 units; n, p, n', and p' are 0 to 2 units; and o, q, m', and o' are 0 to 5.

[0224] In some embodiments, the first wing sequence (A in the formula) comprises a combination of nucleotide analogs and DNA unit selected from (i) 1-9 nucleotide analogs and 1 DNA unit; (ii) 1-8 nucleotide analogs and 1-2 DNA units; (iii) 1-7 nucleotide analogs and 1-3 DNA units; (iv) 1-6 nucleotide analogs and 1-4 DNA units; (v) 1-5 nucleotide analogs and 1-5 DNA units; (vi) 1-4 nucleotide analogs and 1-6 DNA units;

(vii) 1-3 nucleotide analogs and 1-7 DNA units; (viii) 1-2 nucleotide analogs and 1-8 DNA units; and (ix) 1 nucleotide analog and 1-9 DNA units.

[0225] In certain embodiments, the second wing sequence (C in the formula) comprises a combination of nucleotide analogs and DNA unit selected from (i) 1-9 nucleotide analogs and 1 DNA unit; (ii) 1-8 nucleotide analogs and 1-2 DNA units; (iii) 1-7 nucleotide analogs and 1-3 DNA units; (iv) 1-6 nucleotide analogs and 1-4 DNA units; (v) 1-5 nucleotide analogs and 1-5 DNA units; (vi) 1-4 nucleotide analogs and 1-6 DNA units; (vii) 1-3 nucleotide analogs and 1-7 DNA units; (viii) 1-2 nucleotide analogs and 1-8 DNA units; and (ix) 1 nucleotide analog and 1-9 DNA units.

[0226] In some embodiments, region A in the oligomer formula has a sub-formula selected from L, LL, LDL, LLL, LLDL, LDLL, LDDL, LLLL, LLLLL, LLLDL, LLDLL, LDLLL, LLDDL, LDDLL, LLDLD, LDLLD, LDDDL, LLLLLL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LLDDL, LDDLLL, LDLLDL, LDLDLL, LDDDLL, LLDDL, and LDLDLD, and region C in the oligomer formula has a sub-formula selected from L, LL, LDL, LLL, LLDL, LLLL, LDLL, LDDL, LLDD, LLLLL, LLLLD, LLLDL, LLDLL, LDLLL, LLDDL, LDDLL, LLDLD, LDLLD, LDDDL, LLLLLL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LLDDL, LDDLLL, LDLLDL, LDLDLL, LDDDLL, LLDDL, and LDLDLD, wherein at least one of regions A and C comprises at least one DNA nucleoside D.

[0227] In some embodiments, region A in the oligomer formula has a sub-formula selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLLDL, LLDLL, LDLLL, LLDDL, LDDLL, LLDLD, LDLLD, LDDDL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LLDDL, LDDLLL, LDLLDL, LDLDLL, LDDDLL, LLDDL, and LDLDLD, and region C in the oligomer formula has a sub-formula selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLDD, LLLLD, LLLDL, LLDLL, LDLLL, LLDDL, LDDLL, LLDLD, LDLLD, LDDDL, LLLLDL, LLLDLL, LLDLLL, LDLLLL, LLLDDL, LLDLDL, LLDDL, LDDLLL, LDLLDL, LDLDLL, LDDDLL, LLDDL, and LDLDLD.

[0228] In some embodiments, region A in the oligomer has a sub-formula selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLLDL, LLDLL, LDLLL, LLDDL, LDDLL, LLDLD, LDLLD, LDDDL, LLLLDL, LLLDLL, LLDLLL, LDLLLL,

LLLDDL, LLDLDL, LLDDL, LDDL, LDLLDL, LDLDLL, LDDDDL, LLDDL, and LDLDL.

[0229] In some embodiments, region C in the oligomer formula has a sub-formula selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLDD, LLLD, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDL, LLLLDL, LLLDLL, LLDLLL, LDLLL, LLLDDL, LLDLDL, LLDDL, LDDL, LDLLDL, LDLDLL, LDDL, LLDDL, and LDLDL.

[0230] In some embodiments, region A in the oligomer has a sub-formula selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLLD, LLDLL, LDLLL, LDDL, LDDL, LLDLD, LDLLD, LDDL, LLLLDL, LLLDLL, LLDLLL, LDLLL, LLLDDL, LLDLDL, LLDDL, LDDL, LDLLDL, LDLDLL, LDDL, LLDDL, and LDLDL, and region B is a length of 8, 9, 10, 11 or 12 (or 13 or 14) contiguous DNA nucleosides, and region C in the oligomer has a sub-formula selected from the group consisting of LL, LDL, LLL, LLDL, LLLL, LDLL, LDDL, LLDD, LLLL, LLLD, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDL, LLLL, LLLLDL, LLLDLL, LLDLLL, LDLLL, LLLDDL, LLDLDL, LLDDL, LDDL, LDLLDL, LDLDLL, LDDL, LLDDL, and LDLDL.

[0231] In some embodiments, region A in the oligomer formula has a sub-formula selected from the group consisting of L, LL, LDL, LLL, LLDL, LDLL, LDDL, LLLL, LLLL, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDL, LLLL, LLLLDL, LLLDLL, LLDLLL, LDLLL, LLLDDL, LLDLDL, LLDDL, LDDL, LDLLDL, LDLDLL, LDDL, LLDDL, and LDLDL, and region B is a length of 8, 9, 10, 11 or 12 contiguous DNA nucleosides, and region C in the oligomer has a sub-formula selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLDD, LLLD, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDL, LLLLDL, LLLDLL, LLDLLL, LDLLL, LLLDDL, LLDLDL, LLDDL, LDDL, LDLLDL, LDLDLL, LDDL, LLDDL, and LDLDL.

[0232] In some embodiments, region C in the oligomer formula has a sub-formula selected from the group consisting of LDL, LLDL, LDLL, LDDL, LLDD, LLLD, LLLDL, LLDLL, LDLLL, LLDDL, LDDL, LLDLD, LDLLD, LDDL, LLLLDL, LLLDLL, LLDLLL, LDLLL, LLLDDL, LLDLDL, LLDDL, LDDL, LDLLDL, LDLDLL, LDDL, LLDDL, and LDLDL.

[0233] In certain embodiments, the oligomer has the formula of 5' A-B-C 3', wherein region B is a contiguous sequence of 7 to 23 DNA units, such as 8, 9, 10, 11, 12, 13 or 14 DNA units, region A has a formula of LLDLL or LDLLL, and region C has the formula of LLDLL, and wherein L is an LNA unit and D is a DNA unit.

[illegible]

LLDLDDDDDDDDDDDDDL LLL, LLDDLDDDDDDDDDDDDDL LLL,
 LLLDLDDDDDDDDDDDDDL LLL, LLDLDDDDDDDDDDDDDDDL LLL,
 LLDLLDDDDDDDDDDDDDL LLL, LLDDLDDDDDDDDDDDDDDDL LLL,
 LLLDDDDDDDDDDDDDLDDL LLL, LLLDLDDDDDDDDDDDDDDDL LLL,
 LLDLLDDDDDDDDDDDDDDDL LLL, LLLLDDDDDDDDDDDDDLDDL LLL,
 LLLLDDDDDDDDDDDDDLDDL LLL, LLLDDLDDDDDDDDDDDLDDL LLL,
 LLDDLDDLDDDDDDDDDDDL LLL, LLDDLDDDDDDDDDDDLDDL LLL,
 LLLDLDDDDDDDDDDDLDDL LLL, LLDLLDDDDDDDDDDDLDDL LLL,
 LLLDLDDDDDDDDDDDDDLDDL LLL, LLDLDDLDDDDDDDDDDDL LLL,
 LLLLDDDDDDDDDDDLDDL LLL, LLLDLDDDDDDDDDDDLDDL LLL,
 LLDLDDLDDDDDDDDDDDL LLL, LLDLLDDDDDDDDDDDLDDL LLL,
 LLDLDDLDDDDDDDDDDDLDDL LLL, LLDDLDDDDDDDDDDDLDDL LLL,
 LLLLDDDDDDDDDDDLDDL LLL, LLLDDLDDDDDDDDDDDLDDL LLL,
 LLDLDDLDDDDDDDDDDDL LLL, LLDLLDDDDDDDDDDDLDDL LLL,
 LLDLDDLDDDDDDDDDDDLDDL LLL, LLDDLDDDDDDDDDDDLDDL LLL,
 LLLLDDDDDDDDDDDLDDL LLL, LLLDDLDDDDDDDDDDDLDDL LLL,
 LLDLDDLDDDDDDDDDDDLDDL LLL, LLLLDDDDDDDDDDDDDLDDL LLL, and
 LLDDLDDDDDDDDDDDDDLDDL; wherein L represents a LNA nucleoside, and D
 represents a DNA nucleoside. In some embodiments, L represents a beta-D-oxy LND
 nucleoside. It can be recognized that in some embodiments, region B of consecutive DNA
 nucleosides can be varied, e.g. 7, 8, 9, 10, 11, 12, 13 or 14 DNA nucleotides in length.
 The internucleoside linkages of the oligomers of the invention can, for example be
 phosphorothioate internucleoside linkages.

[0235] In some embodiments, the oligomer of the invention has an alternating flank design
 of LNA and DNA nucleoside units, selected from the group consisting of (5' – 3') 2-3-2-
 8-2, 1-1-2-1-1-9-2, 3-10-1-1-2, 3-9-1-2-2, 3-8-1-3-2, 3-8-1-1-1-1-2, 3-1-1-9-3, 3-1-1-8-1-
 1-2, 4-9-1-1-2, 4-8-1-2-2, 3-3-1-8-2, 3-2-1-9-2, 3-2-2-8-2, 3-2-2-7-3, 5-7-1-2-2, 1-1-3-10-
 2, 1-1-3-7-1-2-2, 1-1-4-9-2, 2-1-3-9-2, 3-1-1-10-2, 3-1-1-7-1-2-2, 3-1-2-9-2, 4-7-1-3-2, 5-
 9-1-1-2, 4-10-1-1-2, 3-11-1-1-2, 2-1-1-10-1-1-2, 1-1-3-9-1-1-2, 3-10-1-2-2, 3-9-1-3-2, 3-
 8-1-1-1-2-2, 4-9-1-2-2, 4-9-1-1-3, 4-8-1-3-2, 4-8-1-2-3, 4-8-1-1-1-1-2, 4-7-1-2-1-1-2, 4-
 7-1-1-1-2-2, 2-1-2-11-2, 2-1-3-8-1-1-2, 3-1-1-11-2, 3-1-1-9-1-1-2, 3-1-1-8-1-2-2, 3-1-1-

7-1-1-1-1-2, 4-9-2-1-2, 4-7-1-3-3, 5-9-1-1-3, 5-9-1-2-2, 4-10-2-1-2, 4-10-1-1-3, 4-10-1-2-2, 3-11-2-1-2, 3-11-1-1-3, 5-9-2-1-2, 3-11-1-2-2, 2-1-2-9-1-2-2, 3-1-1-10-1-1-2, 3-1-1-9-1-2-2, 4-9-1-1-1-1-2, 4-8-2-1-1-1-2, 1-1-3-10-2-1-2, 2-1-2-10-2-1-2, 2-1-1-12-4, 2-2-1-11-4, 3-1-1-11-4, 2-1-1-13-3, 2-1-2-11-4, 2-2-1-12-3, 3-11-1-2-3, 3-1-1-12-3, 2-1-2-12-3, 4-11-2-1-2, 4-10-2-2-2, 3-2-1-9-1-1-3, 2-2-1-1-1-9-4, 2-2-2-9-1-1-3, 3-1-1-9-1-1-1-1-2, 2-1-2-9-1-2-3, 3-1-1-10-1-1-3, 2-1-1-2-1-9-4, 4-9-1-1-1-2-2, 3-1-1-9-1-2-3, 2-1-1-1-1-10-4, 2-1-2-10-1-1-3, 2-1-1-1-1-9-2-1-2, 2-2-2-9-2-1-2, 4-9-1-2-1-1-2, 3-2-1-9-2-1-2, 2-1-2-9-2-2-2, 2-1-1-1-1-9-1-1-3, 3-1-1-9-2-2-2, 2-2-2-10-4, 2-1-2-9-1-1-1-1-2, 4-10-1-2-3, 3-2-1-10-4, 3-1-1-10-2-1-2, 4-10-1-1-1-1-2, 4-11-1-1-3, and 2-2-2-10-1-1-2; wherein the first numeral represents the number of LNA units, the next the number of DNA units, and alternating LNA and DNA regions thereafter. The LNA units can for example all be beta-D-oxy LNA units.

[0236] In other embodiments, the oligomers of the invention are gapmers having the formula of 5' A-B-C 3', wherein the oligomer has 12 to 25 nucleotides in length, A is a first wing sequence having the formula of $L_m d_n L_o d_p L_q$, C is a second wing sequence having the formula of $L_q d_p L_o d_n L_m$, wherein each wing independently has 1-17 nucleotides in length and is optionally interrupted by DNA spacers d_n , d_p , d_n , and d_p , each of which independently has 0 to 3 DNA units, with each wing flanking an all DNA gap of 7 to 23 nucleotides; wherein m and m' are at least 1; and n, n', p and p' are independently 0-3 units; such that $m + n + o + p + q = 1-17$; and independently $m' + n' + o' + p' + q' = 1-17$; or $(m + n + o + p + q)$ and $(m' + n' + o' + p' + q')$ are independently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17; or B comprises a DNA gap of 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23;

[0237] Further gapmer designs are disclosed in WO2004/046160, which is hereby incorporated by reference in its entirety. WO2008/113832 hereby incorporated by reference in its entirety, refers to 'shortmer' gapmer oligomers. In some embodiments, oligomers presented herein can be such shortmer gapmers.

[0238] In some embodiments the oligomer comprises a contiguous nucleotide sequence of a total of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotide units, wherein the

contiguous nucleotide sequence is of formula (5' – 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein; A consists of 1, 2, 3, 4, or 5 nucleotide analog units, such as LNA units; B consists of 7, 8, 9, 10, 11, 12, 13, or 14 contiguous nucleotide units which are capable of recruiting RNase when formed in a duplex with a complementary RNA molecule (such as a mRNA target); and C consists of 1, 2, 3, 4, or 5 nucleotide analog units, such as LNA units. When present, D consists of a single DNA unit.

[0239] In some embodiments A comprises 1 LNA unit. In some embodiments A comprises 2 LNA units. In some embodiments A comprises 3 LNA units. In some embodiments A comprises 4 LNA units. In some embodiments A comprises 5 LNA units. In some embodiments C comprises 2 LNA units. In some embodiments C comprises 3 LNA units. In some embodiments C comprises 4 LNA units. In some embodiments C comprises 5 LNA units. In some embodiments B comprises 7 nucleotide units. In some embodiments B comprises 8 nucleotide units. In some embodiments B comprises 9 nucleotide units. In certain embodiments, region B comprises 10 nucleoside units. In certain embodiments, region B comprises 11 nucleoside units. In certain embodiments, region B comprises 12 nucleoside units. In certain embodiments, region B comprises 13 nucleoside units. In certain embodiments, region B comprises 14 nucleoside units. In certain embodiments, region B comprises 7–23 DNA monomers. In some embodiments B comprises from 7–23 DNA units, such as 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 DNA units. In some embodiments B consists of DNA units. In some embodiments B comprises at least one LNA unit which is in the alpha-L configuration, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 LNA units in the alpha-L-configuration. In some embodiments B comprises at least one alpha-L-oxy LNA unit or wherein all the LNA units in the alpha-L-configuration are alpha-L-oxy LNA units. In some embodiments, the oligomer contains 10 DNA units in B, LDLLL in A (first wing) and LLDLL in C (second wing). In yet other embodiments, the oligomer contains 9 DNA units in B, LDDLL in A, LDLDLL in C. In still other embodiments, the oligomer contains 10 DNA units in B, LLDLL in A, and LLDLL in C. In further embodiments, the oligomer contains 9 DNA units in B, LLLLL in A, and LDDLL in C. In certain embodiments, each of regions A and C comprises three LNA monomers, and region B consists of 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 nucleoside monomers, for example, DNA monomers.

II.H. Internucleotide Linkages

- [0240] The monomers of the oligomers described herein are coupled together via linkage groups. Suitably, each monomer is linked to the 3' adjacent monomer via a linkage group.
- [0241] The person having ordinary skill in the art would understand that, in the context of the present invention, the 5' monomer at the end of an oligomer does not comprise a 5' linkage group, although it may or may not comprise a 5' terminal group.
- [0242] The terms "linkage group" or "internucleotide linkage" are intended to mean a group capable of covalently coupling together two nucleotides. Specific and preferred examples include phosphate groups and phosphorothioate groups.
- [0243] The nucleotides of the oligomer of the invention or contiguous nucleotides sequence thereof are coupled together via linkage groups. Suitably each nucleotide is linked to the 3' adjacent nucleotide via a linkage group.
- [0244] Suitable internucleotide linkages include those listed within WO2007/031091, for example the internucleotide linkages listed on the first paragraph of page 34 of WO2007/031091 (hereby incorporated by reference in its entirety).
- [0245] Examples of suitable internucleotide linkages that can be used with the invention include phosphodiester linkage, a phosphotriester linkage, a methylphosphonate linkage, a phosphoramidate linkage, a phosphorothioate linkage, and combinations thereof.
- [0246] It is, in some embodiments, preferred to modify the internucleotide linkage from its normal phosphodiester to one that is more resistant to nuclease attack, such as phosphorothioate or boranophosphate – these two, being cleavable by RNaseH, also allow that route of antisense inhibition in reducing the expression of the target gene.
- [0247] Suitable sulphur (S) containing internucleotide linkages as provided herein may be preferred. Phosphorothioate internucleotide linkages are also preferred, particularly for the gap region (B) of gapmers. Phosphorothioate linkages can also be used for the flanking regions (A and C, and for linking A or C to D, and within region D, as appropriate).
- [0248] Regions A, B and C, can, however, comprise internucleotide linkages other than phosphorothioate, such as phosphodiester linkages, particularly, for instance when the use of nucleotide analogs protects the internucleotide linkages within regions A and C from endo-nuclease degradation – such as when regions A and C comprise LNA nucleotides.

- [0249] The internucleotide linkages in the oligomer can be phosphodiester, phosphorothioate or boranophosphate so as to allow RNaseH cleavage of targeted RNA. Phosphorothioate is preferred, for improved nuclease resistance and other reasons, such as ease of manufacture.
- [0250] In one aspect of the oligomer of the invention, the nucleotides and/or nucleotide analogs are linked to each other by means of phosphorothioate groups.
- [0251] It is recognized that the inclusion of phosphodiester linkages, such as one or two linkages, into an otherwise phosphorothioate oligomer, particularly between or adjacent to nucleotide analog units (typically in region A and or C) can modify the bioavailability and/or bio-distribution of an oligomer – see WO2008/113832, hereby incorporated by reference.
- [0252] In some embodiments, such as the embodiments referred to above, where suitable and not specifically indicated, all remaining linkage groups are either phosphodiester or phosphorothioate, or a mixture thereof.
- [0253] In some embodiments all the internucleotide linkage groups are phosphorothioate.
- [0254] When referring to specific gapmer oligonucleotide sequences, such as those provided herein it will be understood that, in various embodiments, when the linkages are phosphorothioate linkages, alternative linkages, such as those disclosed herein can be used, for example phosphate (phosphodiester) linkages can be used, particularly for linkages between nucleotide analogs, such as LNA, units. Likewise, when referring to specific gapmer oligonucleotide sequences, such as those provided herein, when the C residues are annotated as 5'methyl modified cytosine, in various embodiments, one or more of the Cs present in the oligomer can be unmodified C residues.
- [0255] US Publication No. 2011/0130441, which was published June 2, 2011 and is incorporated by reference herein in its entirety, refers to oligomeric compounds having at least one bicyclic nucleoside attached to the 3' or 5' termini by a neutral internucleoside linkage. The oligomers of the invention can therefore have at least one bicyclic nucleoside attached to the 3' or 5' termini by a neutral internucleoside linkage, such as one or more phosphotriester, methylphosphonate, MMI, amide-3, formacetal or thioformacetal. The remaining linkages can be phosphorothioate.
- [0256] In some embodiments, the oligomers of the invention have internucleotide linkages described in Figure 2, 6B or 11B. As used herein, e.g., figures 2, 6B or 11B,

phosphorothioate linkages are indicated as "s", and phosphorodiester linkages are indicated by the absence of "s."

III. Conjugates

- [0257] In the context the term "conjugate" is intended to indicate a heterogeneous molecule formed by the covalent or non-covalent attachment ("conjugation") of the oligomer as described herein to one or more non-nucleotide, or non-polynucleotide moieties. Examples of non-nucleotide or non-polynucleotide moieties include macromolecular agents such as proteins, fatty acid chains, sugar residues, glycoproteins, polymers, or combinations thereof. Typically proteins can be antibodies for a target protein. In some embodiments, typical polymers are polyethylene glycol.
- [0258] Therefore, in various embodiments, the oligomer of the invention comprises both a polynucleotide region which typically consists of a contiguous sequence of nucleotides, and a further non-nucleotide region. When referring to the oligomer of the invention comprising a contiguous nucleotide sequence, the compound can comprise non-nucleotide components, such as a conjugate component.
- [0259] The invention also provides for a conjugate comprising the oligomer according to the invention as herein described, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to the oligomer. Therefore, in various embodiments where the oligomer of the invention comprises a specified nucleic acid or nucleotide sequence, as herein disclosed, the compound can also comprise at least one non-nucleotide or non-polynucleotide moiety (*e.g.*, not comprising one or more nucleotides or nucleotide analogs) covalently attached to the oligomer.
- [0260] Conjugation (to a conjugate moiety) can enhance the activity, cellular distribution or cellular uptake of the oligomer of the invention. Such moieties include, but are not limited to, antibodies, polypeptides, lipid moieties such as a cholesterol moiety, cholic acid, a thioether.
- [0261] The oligomers of the invention can also be conjugated to active drug substances, for example, aspirin, ibuprofen, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.
- [0262] In certain embodiments the conjugated moiety is a sterol, such as cholesterol.

III.A. Activated oligomers

[0263] The term "activated oligomer," as used herein, refers to an oligomer of the invention that is covalently linked (*i.e.*, functionalized) to at least one functional moiety that permits covalent linkage of the oligomer to one or more conjugated moieties, *i.e.*, moieties that are not themselves nucleic acids or monomers, to form the conjugates herein described. Typically, a functional moiety will comprise a chemical group that is capable of covalently bonding to the oligomer via, *e.g.*, a 3'-hydroxyl group or the exocyclic NH₂ group of the adenine base, a spacer that can be hydrophilic and a terminal group that is capable of binding to a conjugated moiety (*e.g.*, an amino, sulfhydryl or hydroxyl group). In some embodiments, this terminal group is not protected, *e.g.*, is an NH₂ group. In other embodiments, the terminal group is protected, for example, by any suitable protecting group such as those described in "Protective Groups in Organic Synthesis" by Theodora W Greene and Peter G M Wuts, 3rd edition (John Wiley & Sons, 1999).

[0264] In some embodiments, oligomers of the invention are functionalized at the 5' end in order to allow covalent attachment of the conjugated moiety to the 5' end of the oligomer. In other embodiments, oligomers of the invention can be functionalized at the 3' end. In still other embodiments, oligomers of the invention can be functionalized along the backbone or on the heterocyclic base moiety. In yet other embodiments, oligomers of the invention can be functionalized at more than one position independently selected from the 5' end, the 3' end, the backbone and the base.

[0265] In some embodiments, activated oligomers of the invention are synthesized by incorporating during the synthesis one or more monomers that is covalently attached to a functional moiety. In other embodiments, activated oligomers of the invention are synthesized with monomers that have not been functionalized, and the oligomer is functionalized upon completion of synthesis.

IV. Pharmaceutical Compositions and Administration Routes

[0266] The oligomer of the invention can be used in pharmaceutical formulations and compositions. Suitably, such compositions comprise a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

[0267] The oligomer of the invention can be included in a unit formulation such as in a pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a

patient a therapeutically effective amount without causing serious side effects in the treated patient.. However, in some forms of therapy, serious side effects may be acceptable in terms of ensuring a positive outcome to the therapeutic treatment.

[0268] The formulated drug may comprise pharmaceutically acceptable binding agents and adjuvants. Capsules, tablets, or pills can contain for example the following compounds: microcrystalline cellulose, gum or gelatin as binders; starch or lactose as excipients; stearates as lubricants; various sweetening or flavoring agents. For capsules the dosage unit may contain a liquid carrier like fatty oils. Likewise coatings of sugar or enteric agents may be part of the dosage unit. The oligonucleotide formulations can also be emulsions of the active pharmaceutical ingredients and a lipid forming a micellular emulsion.

[0269] The pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be (a) oral (b) pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, (c) topical including epidermal, transdermal, ophthalmic and to mucous membranes including vaginal and rectal delivery; or (d) parenteral including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, *e.g.*, intrathecal, intra-cerebroventricular, or intraventricular, administration. In one embodiment the oligomer is administered IV, IP, orally, topically or as a bolus injection or administered directly in to the target organ. In another embodiment, the oligomer is administered intrathecal or intra-cerebroventricular as a bolus injection.

[0270] Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, sprays, suppositories, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Examples of topical formulations include those in which the oligomer of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Compositions and formulations for oral administration include but are not limited to powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitables. Compositions and formulations for parenteral, intrathecal, intra-

cerebroventricular, or intraventricular administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0271] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Delivery of drug to the target tissue can be enhanced by carrier-mediated delivery including, but not limited to, cationic liposomes, cyclodextrins, porphyrin derivatives, branched chain dendrimers, polyethylenimine polymers, nanoparticles and microspheres (Dass CR. J Pharm Pharmacol 2002; 54(1):3-27).

[0272] The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0273] For parenteral, subcutaneous, intradermal or topical administration the formulation can include a sterile diluent, buffers, regulators of tonicity and antibacterials. The active oligomers can be prepared with carriers that protect against degradation or immediate elimination from the body, including implants or microcapsules with controlled release properties. For intravenous administration the carriers can be physiological saline or phosphate buffered saline. International Publication No. WO2007/031091 (A2), published March 22, 2007, further provides suitable pharmaceutically acceptable diluent, carrier and adjuvants - which are hereby incorporated by reference.

VII. Methods of Using

[0274] The oligomers of the invention can be utilized as research reagents for, for example, diagnostics, therapeutics and prophylaxis.

[0275] In research, such oligomers can be used to specifically inhibit the synthesis of a target protein (typically by degrading or inhibiting the mRNA and thereby prevent protein

formation) in cells and experimental animals thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention. Further provided are methods of down-regulating the expression of a target mRNA and/or a target protein in cells or tissues comprising contacting the cells or tissues, *in vitro* or *in vivo*, with an effective amount of one or more of the oligomers, conjugates or compositions of the invention.

[0276] In diagnostics the oligomers can be used to detect and quantitate target transcript expression in cell and tissues by northern blotting, *in-situ* hybridization or similar techniques.

[0277] For therapeutics, an animal or a human, suspected of having a disease or disorder, which can be treated by modulating the expression of a target transcript and/or target protein is treated by administering oligomeric compounds in accordance with this invention while reducing the toxicity of oligomeric compounds. Further provided are methods of treating a mammal, such as treating a human, suspected of having or being prone to a disease or condition, associated with expression of a target transcript and/or target protein by administering a therapeutically or prophylactically effective amount of one or more of the oligomers or compositions of the invention. The oligomer, a conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount. In some embodiments, the oligomer or conjugate of the invention is used in therapy.

[0278] In certain embodiments, the disease, disorder, or condition is associated with overexpression of the target gene transcript and/or target protein.

[0279] The invention also provides for methods of inhibiting (*e.g.*, by reducing) the expression of target gene transcript and/or target protein in a cell or a tissue, the method comprising contacting the cell or tissue, *in vitro* or *in vivo*, with an effective amount of one or more oligomers, conjugates, or pharmaceutical compositions thereof, of the invention to affect degradation of expression of target gene transcript thereby reducing target protein.

[0280] The invention also provides for the use of the oligomer or conjugate of the invention as described for the manufacture of a medicament for the treatment of a disorder as referred to herein, or for a method of the treatment of as a disorder as referred to herein

- [0281] The invention further provides for a method for inhibiting target protein in a cell which is expressing the target comprising administering an oligomer or a conjugate according to the invention to the cell so as to affect the inhibition of the target protein in the cell.
- [0282] The invention also provides for a method for treating a disorder as referred to herein the method comprising administering an oligomer or a conjugate according to the invention as herein described and/or a pharmaceutical composition according to the invention to a patient in need thereof.
- [0283] Also provided is a method of treating a disease or condition in a subject in need thereof while reducing the toxicity of the treatment comprising administering the oligomer, the conjugate, or the pharmaceutical composition to the subject, wherein the administering treats the disease or condition while reducing the toxicity of the treatment compared to a treatment using a corresponding reference oligomer, which reference oligomer comprises formula G-H-I, wherein region H is identical to region B, and regions G and I comprise the same sequence as regions A and C, but comprises all LNA nucleoside units.
- [0284] The oligomers and other compositions according to the invention can be used for the treatment of conditions associated with over expression or expression of mutated version of target protein.
- [0285] An interesting aspect of the invention is directed to the use of an oligomer (compound) as defined herein or a conjugate as defined herein for the preparation of a medicament for the treatment of a disease, disorder or condition as referred to herein.
- [0286] The invention also relates to an oligomer, a composition or a conjugate as defined herein for use as a medicament.
- [0287] A patient who is in need of treatment is a patient suffering from or likely to suffer from the disease or disorder.
- [0288] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook *et al.*, ed. (1989) *Molecular Cloning A Laboratory Manual* (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) *Molecular Cloning: A Laboratory Manual*,

(Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); Wu *et al.*, eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987) Immunochemical Methods In Cell And Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986);); Crooks, Antisense drug Technology: Principles, strategies and applications, 2nd Ed. CRC Press (2007) and in Ausubel *et al.* (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

[0289] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0290] The following description of the specific embodiments is offered by way of illustration and not by way of limitation. The following examples will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0291] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the claims.

EXAMPLES

Example 1: Construction of Oligomers

- [0292] A number of oligomers were designed to target the 3' UTR of *MAPT* pre-mRNA. For example, the oligomers were constructed to target nucleotides 134,821-138,940 of SEQ ID NO: 1. The exemplary sequences of the oligomers are described in Figures 1, 2, 3, and 4. Figure 1 shows non-limiting examples of the oligomer design for selected sequences. The same methods can be applied to any other sequences disclosed herein. The gapmers were constructed to contain locked nucleic acids – LNAs (upper case letters). For example, a gapmer can have Beta-deoxy LNA at the 5' end and the 3' end and have a phosphorothioate backbone. But the LNAs can also be substituted with any other nucleotide analog and the backbone can be other type of backbone (*e.g.*, a phosphodiester linkage, a phosphotriester linkage, a methylphosphonate linkage, a phosphoramidate linkage, or combinations thereof).
- [0293] The oligomers were synthesized using methods well known in the art. Exemplary methods of preparing such oligomers are described in Barciszewski *et al.*, Chapter 10 – "Locked Nucleic Acid Aptamers" in *Nucleic Acid and Peptide Aptamers: Methods and Protocols*, vol. 535, Gunter Mayer (ed.) (2009), the entire contents of which is hereby expressly incorporated by reference herein.
- [0294] In Figure 1, in the Sequence designation, upper case designates a modified nucleotide such as an LNA nucleotide (either Beta-D-Oxy, Alpha-L-Oxy, Beta-D-Amino or Beta-D-Thio LNA or other modified nucleotide such as cEt, cMOE, UNA or ENA) and lower case designates a DNA nucleotide. Thus a sequence represented by TCCCTtaatttcacCCtCA (SEQ ID NO. 29) represents a 5-9-2-1-2 19mer, *e.g.*, LLLLLDDDDDDDDDDLLDLL. Selected examples of alternating flank gapmers having a 7 nucleotide gap are ASO-002399, ASO-002482, ASO-002437, and ASO-002425. Any one of the oligomer sequences disclosed herein can have the chemical structure (design) of the alternating flank gapmer design shown in the figures.
- [0295] In Figure 1, the following designate the components of the oligonucleotides of the present invention, with oligonucleotides always depicted in the 5' to 3' direction. Therefore, the 5' end of an oligomer hybridizes to the pre-mRNA end number in the figure and the 3' end of the oligomer hybridizes to the pre-mRNA start number in the

figure. A reference to a SEQ ID number includes a particular sequence, but does not include an oligomer design.

- [0296]** Beta-D-oxy LNA nucleotides are designated by OxyB where B designates a nucleotide base such as thymine (T), uridine (U), cytosine (C), methylcytosine (MC), adenine (A) or guanine (G), and thus include OxyA, OxyT, OxyMC, OxyC and OxyG.
- [0297]** Alpha-L-oxy LNA nucleotides are designated by AlfaOxyB where B designates a nucleotide base such as thymine (T), uridine (U), cytosine (C), methylcytosine (MC), adenine (A) or guanine (G), and thus include AlfaOxyA, AlfaOxyT, AlfaOxyMC, AlfaOxyC and AlfaOxyG. The letter M or m before C or c indicates 5-methylcytosine.
- [0298]** Beta-D-Amino LNA nucleotides are designated by AminoB where B designates a nucleotide base such as thymine (T), uridine (U), cytosine (C), methylcytosine (MC), adenine (A) or guanine (G), and thus include AminoA, AminoT, AminoMC, AminoC and AminoG. The letter M or m before C or c indicates 5-methylcytosine.
- [0299]** Beta-D-Thio-LNA nucleotides are designated by ThioB where B designates a nucleotide base such as thymine (T), uridine (U), cytosine (C), methylcytosine (MC), adenine (A) or guanine (G), and thus include ThioA, ThioT, ThioMC, ThioC and ThioG. The letter M or m before C or c indicates 5-methylcytosine.
- [0300]** DNA nucleotides are designated by DNAb, where the lower case b designates a nucleotide base such as thymine (T), uridine (U), cytosine (C), 5-methylcytosine (MC), adenine (A) or guanine (G), and thus include DNAa, DNAt, DNAc, DNAmc and DNAG. The letter M or m before C or c indicates 5-methylcytosine. Some examples of the oligomers containing 5 methyl cytosine include, but are not limited to, ASO-002658, ASO-002629, ASO-002621, and ASO-002665.
- [0301]** The letter "s" after the nucleotide designation indicates phosphorothioate linkage whereas absence of "s" indicates phosphodiester linkage.
- [0302]** Thus a 3-10-3 beta-D-oxy LNA-DNA- beta-D-oxy LNA gapmer with sequence ATTtccaaattcaCTT, with full phosphorothioate internucleotide linkages would be designated OxyAs OxyTs OxyTs DNAts DNACs DNACs DNAAs DNAAs DNAAs DNAts DNAts DNACs DNAAs OxyMCs OxyTs OxyT. In some embodiments, the oligomers include a mix of phosphorothioate or phosphodiester internucleotide linkages. Non limiting examples of the oligomers having a mix of phosphorothioate or phosphodiester internucleotide linkages include ASO-002640, ASO-002632, ASO-002647, ASO-002666,

ASO-002659, ASO-002652, ASO-002645, ASO-002638, ASO-003270, ASO-003269, ASO-003268, ASO-002673, ASO-002661, ASO-002654, and ASO-002668.

Preparation of Oligos with Mismatches

[0303] Oligos having mismatched bases at different locations were also prepared using standard methods well known in the art. Examples of oligomers with mismatched bases are provided in Figure 1 or 2 as "mm." The specific mismatched basepair are bolded, underlined, italicized, and highlighted.

Example 2: *In Vitro* Reduction in Tau Protein

[0304] Each of the oligomers targeting the 3' UTR of an *MAPT* transcript was tested for its ability to decrease Tau protein in mouse primary neurons expressing the entire human *MAPT* gene as a bacmid containing transgene (C57-bl6 BAC-Tg hTau; Polydoro et. al., *J. Neurosci.* (2009) 29 (34): 10747-9). Primary hTau mouse embryonic forebrain neuronal cultures do not express endogenous mouse tau as mouse tau was knocked out. Primary neurons were generated by papain digestion according to manufacturer's protocol (Worthington Biochemical Corporation, LK0031050). Briefly, forebrains were dissected from hTau mouse E18 BAC-Tg embryos expressing the entire human microtubule-associated protein Tau (*MAPT*) gene on a murine *MAPT*-null background and were incubated at 37°C for 30-45 minutes in papain/DNase/Earle's balanced salt solution (EBSS) solution. After trituration and centrifugation of cell pellet, the reaction was stopped by incubation with EBSS containing protease inhibitors, bovine serum albumin (BSA) and DNase. The cells were trituated and washed with Neurobasal (NB, Invitrogen) supplemented with 2% B-27, 100 µg/ml penicillin, 85 µg/ml streptomycin, 0.5mM glutamine. The cells were plated in supplemented NB media onto poly-D-lysine-coated 96-well optical imaging plates (BD Biosciences) at 15,000 cells/well.

[0305] After obtaining the primary hTau mouse embryonic forebrain neuronal cultures expressing a human *MAPT* gene, the cultures were treated with oligomers to inhibit the Tau mRNA and protein expression. The cultures were then subject to immunocytochemistry and imaging to measure the inhibition. One day post plating (DIV 1), half of the supplemented neurobasal (NB) media on the primary hTau mouse embryonic forebrain neuronal cultures was removed and replaced with supplemented NB

media containing various concentrations of LNA oligomers. Primary hTau neuronal cultures were cultured with LNA oligomers until 13 days post plating (DIV 13). On DIV 13, the cultures were rinsed with Dulbecco's phosphate-buffered saline lacking calcium and magnesium (DPBS, Invitrogen) and fixed in 4% paraformaldehyde/4% sucrose/DPBS for 15 min. Cultures were rinsed and then blocked and permeabilized in DPBS plus 0.1% Triton X-100 (TX-100) and 3% BSA for one hour at room temperature. Cultures were rinsed and then incubated for two hours at room temperature with primary antibody 1:500, Tau5 antibody to measure Tau protein, Invitrogen AHB0042; and 1:500, β -III tubulin (TuJ-1) antibody to measure neurite area, Abcam ab41489) in DPBS plus 3% BSA and 0.1% TX-100. Cultures were rinsed and incubated with Hoechst 33342 nuclear dye (1:800, Invitrogen) and AlexaFluor fluorescence-conjugated secondary antibodies (Invitrogen, 1:500) in DPBS plus 3% BSA and 0.1% TX-100 for one hour at room temperature. Cultures were rinsed abundantly and stored in DPBS until imaging. Imaging was conducted using the Cellomics VTi automated immunofluorescence imaging system. In brief, using untreated wells, saturation levels for each fluorophore channel were set to 70%. Then 12 sequential images were acquired from each well, and total fluorescence intensity and total fluorescence area were calculated for both Tau and TuJ-1 proteins using the Cellomics VTi SpotDetector (version 4) image analysis software. To evaluate Tau protein reduction resulting from oligomer treatment, a Tau5 total fluorescence intensity-to-TuJ-1 total fluorescence area ratio (Tau/TuJ-1) was created for each well and then all data were normalized to the average Tau/TuJ-1 ratio of the untreated wells. To evaluate neurite/neuronal toxicity from oligomer treatment, the TuJ-1 total fluorescence area from each well was normalized to the average TuJ-1 total fluorescence area of the untreated wells. TuJ-1 intensity acts as an internal standard for each sample. Nuclei counts from each well were also acquired as an alternative measure of toxicity associated with LNA oligomer treatment. Data are expressed as mean $\pm$ S.D. For immunocytochemistry, data points represent the mean $\pm$ S.D. from wells treated in triplicate. Potency values were generated using wells treated with a broad concentration range of LNA oligomer, from which the resulting normalized Tau/TuJ-1 and TuJ-1 values were analyzed compared to normalized values from saline control samples. Analysis was done using non-linear regression with top and bottom values set at fixed values of 100% and 0%, respectively, where 100% inhibition represents a complete reduction of signal

compared to the control sample (Figure 2). For qPCR, data were analyzed using a one-way ANOVA with a Dunnett's multiple comparison test to compare saline- and LNA oligomer-treated groups. Statistical significance was set at a value of $p < 0.05$.

- [0306] The reduction of Tau protein by each oligomer was compared with saline. The results of the Tau protein reduction compared to Saline are shown in Figure 2. If the Tau protein level in antisense oligonucleotide treated neurons was equal to or higher than in control cells, percent inhibition is expressed as zero inhibition. If present, 'N.D.' indicates 'not determined' and 'TBD' indicates 'to be determined'. The target regions to which antisense oligomers are inhibitory are considered 'hot-spots' on the Tau transcript.
- [0307] Oligomers were diluted in water and added to cells at 1 day post plating (DIV01) to a final concentration of 5 μM . For IC_{50} determinations, neurons were treated with a top concentration of 5 μM and a concentration response dilution of 1:3 was used to define the IC_{50} value. The calculated IC_{50} value for certain oligomers is shown in Figure 3.

Example 3: Spontaneous Calcium Oscillation Measurement

- [0308] The present application shows that a reduction of oscillations in intracellular free calcium concentration (calcium oscillation) corresponds to increased neurotoxicity of an oligomer to a cell. The amount of reduction and how it corresponds to an increase in neurotoxicity can be determined as described herein. To measure primary cortical neuron spontaneous calcium oscillation, rat primary cortical neurons were prepared from Sprague-Dawley rat embryos (E19). Cells were plated 25,000 cells/well onto 384 well poly-D-lysine coated fluorescent imaging plate reader (FLIPR) plates (Greiner Bio-One) in 25 μl /well Neurobasal media containing B27 supplement and 2 mM glutamine. Cells were grown for 11 days at 37°C in 5% CO_2 and fed with 25 μl of additional media on DIV04 and DIV08 for use on DIV11. On the day of the experiment, media was removed from the plate and the cells were washed once with 50 μl /well of 37°C assay buffer (Hank's Balanced Salt Solution with 2 mM CaCl_2 and 10 mM HEPES pH 7.4). Oscillations were tested in the presence and absence of 1mM MgCl_2 . Cells were loaded with a cell permanent fluorescent calcium dye, fluo-4 AM (Life Technologies). Fluo-4 AM was prepared at 2.5 mM in DMSO containing 20% pluronic F-127 then diluted 1:1000 in assay buffer. Cells were incubated 1 hr with 20 μl of 2.5 μM fluo-4 AM at 37°C in 5% CO_2 . After 1 hr 20 μl of room temperature assay buffer was added and the cells

were allowed to equilibrate to room temperature for 10 additional minutes and placed in the FLIPR. Baseline signal (measurement of intracellular calcium) was read for 100 seconds (1 reading/second) before the addition of anti-sense oligomers. Oligomers were added with a 384 well head in the FLIPR in 20 μ l of assay buffer at 75 μ M for a final concentration of 25 μ M. FLIPR signal was read for an additional 200 seconds (1 reading/second) after the addition of oligomer. A second 5 minute post addition plate read (300 one second points) on the FLIPR was conducted to allow for additional data capture. Raw data from 5 minute read was exported and, using Excel, spike amplitude and frequency was calculated. Calculations were performed by measuring the average FLIPR signal over the 300 second read for control (non-treated) wells. For treated wells, a scoring system was developed where a score of 1 was given for each 1 second read where signal increase greater than 50% of the average control value (calculated above). A score of 0 was given for each 1 second read that increase less than 50% of average control value. For each treatment a total score was calculated and converted to percent control for graphical purposes. If the antisense oligomer produced a calcium oscillation response greater than that of AMPA alone, percent of control is expressed as greater than 100% (Figure 3).

- [0309] Effect of oligomers on primary neuronal spontaneous calcium oscillations was measured under two conditions, in the presence and absence of 1mM MgCl_2 as a source of Mg^{2+} ions, as described previously (Murphy et.al., *J. Neurosci.* **12**, 4834 – 4845 (1992)). This was done to isolated N-methyl-D-aspartate (NMDA)- and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- receptor mediated calcium oscillations.
- [0310] Antisense oligomer inhibition of spontaneous calcium oscillations mediated by either NMDA or AMPA was assessed in the presence or absence of 1mM MgCl_2 (representing 100% control in each case). Addition of 25 μ M antisense oligomers (ASO) inhibited AMPA receptor but not NMDA receptor mediated oscillations. ASO, and other oligos that behaved similarly, were shown to negatively impact central nervous system (CNS) network activity *in vivo* and electrophysiologic spontaneous neuronal activity *in vitro* (data not shown). Tau antisense oligonucleotide impact on spontaneous calcium oscillations in primary neurons is summarized in Figure 3. See Murphy, et al., *J. Neurosci.* **12**, 4834 – 4845 (1992).

[0311] Calcium oscillation reduction was measured for the oligomers of the invention and summarized in Figure 3. The oligomers showing greater than 25% of control in the calcium oscillation assay were selected for further analysis.

Example 4: Sequence Score Calculation

[0312] The present application also shows that the sequence score of an oligomer, as calculated herein, corresponds to the neurotoxicity of the oligomer. In certain aspects of the invention, the higher the sequence score the less neurotoxic the oligomer. Different cut off values, over which the sequence score indicates that the oligomer has reduced neurotoxicity, can be determined as described herein.

[0313] The sequence score of each oligomer was calculated to predict the suitability and neurotoxicity of the oligomers. Sequence score is a mathematical calculation determined for all oligomers and is based on the percent of G and C nucleotides, or analogs thereof, within a given oligomer sequence. The following formula was applied to all oligomers in order to calculate sequence score:

$$\frac{\text{number of C nucleotides} - \text{number of G nucleotides}}{\text{nucleotide length}} \quad (I)$$

[0314] An example calculation is given for oligomer ASO-001967 (SEQ ID NO: 188):
TTtATttccaaattcACtTT: $4-0/20 =$ sequence score of 0.20

[0315] The sequence score of the selected oligomers were calculated for further studies. To determine the cut off value for the sequence score, an *In vivo* tolerability study was performed as shown in Example 5.

Example 5: *In Vivo* Tolerability

[0316] The *in vivo* tolerability of the oligomers was tested to see how the oligomer was tolerated when injected into an animal. The *in vivo* tolerability of the oligomers allows for a prediction of how the oligomer will be tolerated when administered therapeutically to a subject such as a human.

Subjects

[0317] *In vivo* tolerability of the oligomers were tested in mice and rats. Animals for Tau qPCR and behavioral studies were adult, C57Bl/6J female mice (20-30g; Jackson

Laboratories, Bar Harbor, ME) housed 3-4 per cage. Animals were held in colony rooms maintained at constant temperature ($21 \pm 2^{\circ}\text{C}$) and humidity ($50 \pm 10\%$) and illuminated for 12 hours per day (lights on at 0600 hours). In some cases, male and female transgenic mice (30-40g) expressing a tau transgene derived from a human PAC, H1 haplotype driven by the tau promoter (Polydoro et. al., J. Neurosci. (2009) 29(34): 10741-9), and in which the native mouse Tau gene was deleted, were used to assess pharmacodynamic endpoints and tissue drug concentrations. For intrathecal infusion studies, female Sprague-Dawley rats (180-225 g at testing; Harlan) were singly housed in colony rooms maintained at a constant temperature ($21 \pm 2^{\circ}\text{C}$) and humidity ($50 \pm 10\%$) and illuminated for 12 hours per day (lights on at 0600 h). All animals had ad libitum access to food and water throughout the studies. Behavioral studies were conducted between 0700 and 1500 hours. Animals were maintained in accordance with the guidelines of the Animal Care and Use Committee of the Bristol-Myers Squibb Company, and the "Guide for Care and Use of Laboratory Animals" published by the National Institutes of Health. Research protocols were approved by the Bristol-Myers Squibb Company Animal Care and Use Committee.

Administration Routes -Intra-cerebroventricular or Intrathecal Injections.

[0318] The oligomers were administered to mice by either intracerebroventricular (ICV) injection or intrathecal injection. Intracerebroventricular injections were performed using a Hamilton micro syringe fitted with a 27 or 30-gauge needle, according to the method of Haley and McCormick. The needle was equipped with a polyethylene guard at 2.5 mm from the tip in order to limit its penetration into the brain. Mice were anesthetized using isoflurane anesthetic (1.5-4%). The mouse to be injected, weighing 20-30g, was held by the loose skin at the back of the neck with the thumb and first fingers of one hand. Applying gentle but firm pressure, the head of the animal was then immobilized by pressing against a firm flat level surface. The needle tip was then inserted through the scalp and the skull, about 1 mm lateral and 1 mm caudal to bregma. Once the needle was positioned, antisense oligonucleotide was given in a volume of 5 microliters in saline vehicle and injected into the right (or left) lateral ventricle over 20-30 seconds. The needle was left in place for 10 seconds before removal. This procedure required no surgery or incision. Animals were warmed on heating pads until they recovered from the procedure. Brain tissue (right, frontal cortical region) was collected on dry ice or

RNA later for drug concentration analysis and Tau qPCR respectively at multiple time points following dosing, *e.g.*, 1 week through 16 weeks post-dosing.

[0319] For intrathecal (IT) injections of mice, animals were maintained under light isoflurane anesthesia (1.5-5%). The mouse was held securely in one hand by the pelvic girdle and inserting a 30G 1/2 inch needle connected to a Hamilton syringe into the tissue between the dorsal aspects of L5 and L6, perpendicular to the vertebral column. When the needle enters the subarachnoid space, a sudden lateral movement of the tail was observed. This reflex was used as an indicator of successful placement of the needle for IT administration. A 5-10 μ L volume of antisense oligonucleotide was injected slowly (over approximately 60 seconds) into the subarachnoid space.

[0320] For intrathecal injections in rat, intrathecal catheters were surgically implanted using methods described by Yaksh and Rudy, *Physiol. Behav.* (1976) 17(6): 1031-6. The rat was mounted to a stereotaxic frame with isoflurane anesthesia maintained through a nose cone. A skin incision was made beginning approximately at the line joining the ears and extending caudally about 3 cm along the midline. The muscle where it attached to the occipital crest of the skull was cut about 3 mm lateral on both sides of the muscle midline. Using retractors or forceps, the muscle was peeled caudally to expose the cisternal membrane at the base of the skull. The fascia and tissue were carefully removed from the membrane. The bent beveled end of a 16-22 gauge needle was used to make a 1-2 mm lateral incision in the cisternal membrane. A sterilized IT catheter, made of polyethylene tubing (PE10 tubing stretched to approximately 1.3 mm outer diameter), was inserted through the incision and carefully advanced caudally through the subarachnoid space while it was rotated between thumb and forefinger and while the base of the tail was gently pulled to align the spinal cord using the other hand. If any resistance was encountered, the catheter was retracted slightly, and slowly advanced again. Once the catheter had been advanced to the desired area, it was flushed with 20 μ L sterile saline and the cranial end was passed through the skin using a 19 gauge needle about 1 cm from the incision. The catheter was plugged with a pin. Rats were given oral antibiotics for 5 days following the surgery. At least five days after surgery, a single antisense oligonucleotide injection was diluted in water and delivered via a programmable infusion pump (Knopf) at a rate of 10 μ L/minute in a volume of 10 to 50 μ L. A brief saline flush of 5 μ L was given just prior to the antisense oligonucleotide delivery and a 10 μ L saline flush

was given just following the oligonucleotide delivery at a rate of 10 µl/minute to cover the dead volume of the catheter (6-7µl). A saline flush of 20ul was also given to animals 1-2X/week until used for an experiment.

- [0321] Data was analyzed using the delta delta Ct method where each sample is first normalized to GAPDH and then expressed as percent of untreated control animals.

Acute Tolerability Behavioral Assessments

- [0322] For one hour following the single injection of antisense oligonucleotide ICV (intra-cerebroventricular) or IT (intrathecal), animals were observed for behavioral side effects and scored for the severity of side effects on a scale of zero (no side effects) to 20 (convulsions resulting in euthanasia). The tolerability scale was divided into 5 neurobehavioral categories: 1) hyperactivity 2) decreased activity and arousal 3) motor dysfunction /ataxia 4) abnormal posture and breathing and 5) tremor/convulsions. Each category was scored on a scale of 0-4, with the worst possible total score of 20. Animals were observed for changes in behavior in the home cage, and then they were removed from the home cage for more detailed observations which included measurement of grip strength and righting reflex.

Novel object recognition

- [0323] Short term recognition memory was measured using the novel object recognition (NOR) task. NOR testing was based on the spontaneous behavior of rodents to explore a novel object more than a familiar one (Dodart et. al., *Neuroreport* (1997) 8(5): 1173-8; Ennaceur and Delacour, *Behav. Brain Res.* (1988) 31 (1):47-59). After a one hour retention interval between training (T1) and testing (T2) sessions, mice remembering the objects from the training session will show a preference for the novel object on the test session. For these experiments, animals were handled for 3 days and habituated to the chamber (48 cm x 38 cm x 20 cm) on the day prior to the test session. The chamber was made of polyethylene and lined with vinyl flooring. On the test day, animals were placed in the rectangular test chamber and allowed to explore two identical objects (7.6 cm high x 5.1 cm wide) for a 15 minute training period. One hour later, mice were placed back into the test chamber for a 10 minute test session, this time with one object they had observed during training and one novel object. Objects were cleaned thoroughly with 25% ethanol between training and testing sessions and between subjects, and were

cleaned again at the end of the day with mild detergent. Object exploration was only considered when the animal's nose was pointed at the object. Exploration was recorded using ObjectScan tracking software (Cleversys, Reston, VA). Data are reported as percent of time spent exploring objects (*i.e.*, novel time/novel+familiar time *100).

Morris Water Maze

[0324] Spatial learning and memory was assessed based on Morris Water Maze assay (Morris *J. Neurosci.* (1984) 11(1):47-60). Water maze represents a pool with the diameter of 120 cm. Water was made opaque using white, non-toxic tempura paint ($20^{\circ}\text{C}\pm 1$). The pool was surrounded with distinct extra-maze cues.

[0325] Prior to hidden platform training, all mice were exposed to the water maze pool by allowing them to swim down the rectangular channel during 2 pre-training trials. The escape platform was placed in the middle of the channel. If a mouse was not able to find and mount the platform during 60 sec trial, it was guided to it and allowed to sit for up to 10 sec. After pre-training, mice underwent hidden platform training, during which a 10x10 cm platform was submerged 1.5 cm below the surface. The platform location remained the same throughout training whereas the drop location varied randomly between the four daily trials as well as across the 4 days of training. Mice received 2 sessions per day for 4 consecutive days. Each session consisted of 2 trials with a 10-min inter-trial interval. The maximum time allowed per trial was 60 sec. If a mouse did not find or mount the platform, it was guided to the platform by the experimenter. All mice were allowed to sit on the platform for 10 sec after each training trial.

[0326] For probe trials, the platform was removed and each mouse was allowed to swim for 60 sec. The drop location for the probe trials was 180° from the platform location used during hidden platform training. After 60 sec, mice were guided to the platform location before retrieval from the pool. For early memory retrieval mice were probed 2h after the last hidden platform training; long term memory recall was assessed 16h following the last hidden platform training. 2h following the 16h probe trial, all mice underwent the visible platform training, where a local cue (pole built using legos) was placed above the hidden platform. Mice were given 2 training trials. All behavior was recorded with a video tracking system (Cleversys Inc). Escape latencies, distance traveled, swim paths, swim speeds, and platform crossings were recorded automatically for subsequent analysis.

Catwalk

- [0327] The Catwalk (Noldus, The Netherlands) is an automated and computerized gait-analysis technique that allows objective quantification of multiple static and dynamic gait parameters. Mice were placed on one end of the catwalk and allowed free exploration for 3 min or until they have 5 compliant trials, whichever comes first. Data were exported and classified using the Catwalk software. An average of classified trials was used for data analysis. Measures of interest include but are not limited to: print position or the distance between the position of the hind paw and previous placement of the ipsilateral front paw, initial and terminal dual stances, paw swing speed, and paw stand or the duration of paw contact with the glass plate in a step cycle.

Behavioral statistics

- [0328] Statistical analyses for all behavioral tests were conducted using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). For NOR, data were analyzed using either a paired t-test for within-group analyses or by an ANOVA followed by a Dunnett's post-hoc test for between group analyses. For MWM, a repeated MWM ANOVA was used to analyze the acquisition phase and a one-way ANOVA followed by Dunnett's post-hoc for probe trial analyses.

Brain Tau mRNA Analysis

Brain homogenization

- [0329] Mouse brain tissue was homogenized in a 10x volume of a high salt /sucrose buffer (10 mM Tris-HCl, pH 7.4, 800 mM NaCl, 10% sucrose (w/v), 1 mM EGTA) supplemented with phosphatase inhibitor cocktail sets 2 and 3, 1 mM PMSF (Sigma, Saint Louis, MO), and complete protease inhibitor cocktail EDTA-free (Roche, Indianapolis, IN) using a Qiagen TissueLyzer II. The homogenate was centrifuged at 20,000 x g for 20 minutes at 4°C. The supernatant was centrifuged at 100,000 Xg for 1 hour at 4°C and the supernatant was analyzed.

RT-PCR assays

- [0330] For cDNA synthesis and subsequent PCR, 300ng of RNA was added to 1 well of a 96 well plate (Axygen, PCR-96-C-S). To each well 7.5µl of master mix (5µL of 2.5mM NTP mix and 2.5µL random primers per reaction) was added and the plate was

centrifuged at 1000 rpm and placed in thermocycler for 3 min at 70° C. Plates were immediately cooled on ice and 4 µl of reaction master mix was added. Prior to PCR, plates were briefly centrifuged to collect sample in bottom of well. cDNA synthesis was carried out at 42° C for 60 min, 95° C for 10 min followed by a hold at 4° C. cDNA Samples were diluted 1:3 with molecular biology grade water and stored at -20° C until further use.

[0331] For PCR, each sample was run in triplicate with two probe sets (MAPT: Taqman Expression assays Hs00902193_m1; RhoA: Taqman Expression assays; GAPDH Taqman Expression assays Hs01922876_u1). To each reaction 4 µl of previously diluted cDNA and 6 µL of master mix was added and plates were centrifuged. Samples were incubated at 95° C for 20 sec follow by 40 cycles at 95° C for 1 sec and 60° C for 20 sec.

Results

[0332] *In vivo* cumulative tolerability threshold following an ICV injection of 100 µg of an antisense oligonucleotide was set at 4. The correlation analysis shows that the oligomers having *in vivo* tolerability lower than 4 tend to have a sequence score equal to or higher than 0.2. Potent LNA oligonucleotides targeting MAPT at or partially overlapping nucleotides in the 3'UTR were identified and found to be well tolerated in primary neurons *in vitro* and following i.c.v. administration *in vivo* (See Figure 4 below).

[0333] The *In vivo* acute tolerability score and brain tau mRNA % control data shown in Figure 4 show that oligomers that hybridize to target MAPT mRNA sequences contained between 134,947-138,924 further identify validated sequence target site oligomers that are both well tolerated and potentially reduce Tau mRNA *In vivo*.

Example 6: Oligomer Prioritization

[0334] The assays described herein can be used in combination to selected oligomers for further testing. Properties of selected oligomers can be described as shown in Table 1. Based on these criteria, certain oligomers were selected for additional dose-response testing *in vitro* and *in vivo*.

Table 1: Summary of criteria used to prioritize oligomers for additional testing.

Assay	Prioritization Criteria
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Tau protein reduction	>70% reduction in Tau protein (5 μ M oligomer)
Calcium oscillations	<25% reduction in calcium oscillations
Sequence score	Sequence score $\geq$ 0.20

[0335] In other embodiment, oligomers can be selected based on the following characteristics: (1) Tau protein reduction >30% reduction in Tau protein (5 μ M oligomer); (2) calcium oscillations <25% reduction in calcium oscillations; and (3) sequence score equal to or higher than 0.2.

Example 7: *In Vivo* Data

[0336] Oligomers were injected into animals to determine their effect on Tau expression and on the behavioral properties of the animal.

Research animals and Administration Routes

[0337] The animals used in this Example are the same mice and rats described in Example 5 and were handled in the same manner as described in Example 5. Animals were injected as described in Example 5.

[0338] RT-PCR assays were performed as described in Example 5.

Running Wheel Assay

[0339] The Home Cage Running Wheel assay measures spontaneous activity in a voluntary free-spinning running wheel (Columbus Instruments). Each wheel has a magnetic sensor that connects to a computer interface and records wheel revolutions at user-specified intervals. In this study, mice were placed individually into cages with a running wheel and wheel rotations were monitored continuously in 15 min increments. To allow for habituation and establish baseline activity levels, control and test mice were tested over 7 days, after which they were transferred into clean cages and dosed with either saline or 100ug of an oligomer by ICV injection. Two weeks post treatment mice were returned to the running wheel cages to evaluate treatment effects over 7 days.

Brain Tau mRNA Analysis

Brain homogenization

Mouse brain tissue was homogenized as described in Example 5. CSF Collection:

[0340] All animal protocols were approved by the Wallingford BMS Animal Care and Use Committee. CSF was collected from the cisterna magna of mice following exsanguination as described by Barten, *et al.*, *J Alz. Res.* 24: 127-141 (2011). In brief, CSF was collected with a P20 pipettor after puncturing the dura with a 30 gauge needle under a dissecting microscope. Body temperature was monitored and maintained at normal levels using heating pads and lamps. CSF was collected from rats after exposure of the cisterna magna and withdrawal using a 1 ml insulin syringe. CSF was placed on ice, centrifuged briefly to remove any red blood cells, transferred to another tube while measuring the volume, and frozen on dry ice. CSF Tau protein reduction measured by Tau Protein Enzyme-Linked ImmunoSorbant Assay (ELISA described below) was observed after 4 weeks following a single bolus i.c.v. injection of an oligomer (data not shown).

Tau Protein Enzyme-Linked ImmunoSorbant Assay (ELISA):

[0341] For brain tissue, BT2 (antibody to Tau amino acid 194-198, Thermo Scientific) was used to coat 96 well black ELISA plates (Costar) at a concentration of 2.5 µg/ml for 1 hour at 37°C. After washing in TBST, the plates were blocked with 3% bovine serum albumin in TBS. Recombinant human Tau441 (rPeptide; Bogart, GA) or a 1:5000 dilution of the brain homogenates were diluted in 1% BSA + 0.05% Tween-20 in TBS. Alkaline phosphatase conjugated Tau-5 (antibody to Tau amino acid 210-230, Covance, Emeryville, CA) was added to the samples at a 1:2000 dilution for co-incubation overnight at 4°C with shaking. After washing in TBST, the signal was amplified with the Tropix CDP Star detection reagent from Applied Biosystems. The chemiluminescent signal was read on an Envision (Perkin Elmer). For CSF samples, this ELISA was done in a 384 well format to minimize the volume of CSF needed. 10 µl of a 1:2 dilution of CSF was added to each well.

[0342] *In vivo* reduction of human tau mRNA level was measured in mice (Figure 4). Brain Tau mRNA and Tau protein reduction was measured over time following a single i.c.v. bolus of 100µg oligomer administration into wild type C57 mice (N=12). Tau mRNA expression (normalized to GAPDH) was measured at 2, 4, 8 and 12 weeks post

injection. Tau protein (% of saline) level was measured at 2, 4, 8 and 12 weeks post injection. This oligomer produced a durable reduction in Tau mRNA and protein with Tau protein remaining reduced following 12 weeks post single bolus *i.c.v.* injection. Other oligomers defined within this invention exhibit more profound reductions in Tau mRNA and protein with durable tissue oligomer exposure as measured by ELISA (further described below).

[0343] In-situ Hybridization (ISH) detection of Tau mRNA and the oligomers was performed on 20 um fresh frozen brain sections mounted. Slides were thawed, fixed in 4% paraformaldehyde for 10 minutes at room temperature, washed in phosphate buffered saline (PBS) and acetylated with 0.25% acetic anhydride/0.1M triethanolamine for 10 minutes at room temperature (RT). Following PBS washes, each slide was pre-hybridized in 0.7 ml pre-warmed hybridization buffer (HB), 50% formamide/5X saline sodium citrate (SSC), 100 µg/ml yeast tRNA, 1X Denhardt's, for 30 minutes at 67°C. 5' FAM-labeled sense probe (complementary all LNA probe) was heated to 90°C for 4 minutes, cooled on ice then diluted in HB. Slides were hybridized in 0.45 ml for 30 minutes at 67°C with a hybridlip (Electron Microscopy Sciences, Hatfield, PA). They were subsequently dipped in 0.1X SSC then washed three times in 0.1X SSC at 67°C. Slides were then treated in 3% hydrogen peroxide for 10 minutes, washed in PBS, and blocked for 15 minutes at RT in 0.1M Tris-HCl, pH 7.5, 0.15M NaCl, 0.5% blocking agent (FP1020, Perkin Elmer Waltham, MA). This was followed by incubation in rabbit anti-fluorescein-horse radish peroxidase for 30 minutes at RT. Following TBST washes (Tris buffered saline with 0.05% Tween 20), tyramide signal amplification was performed (TSA Plus, Perkin Elmer). Slides were washed in TBST, nuclei stained using DAPI, and coverslip mounted using Prolong Gold (Invitrogen, Carlsbad, CA). For chromogenic detection, slides (post-TSA washes) were incubated for a second time with anti-fluorescein-HRP for 30 minutes at RT, washed in TBST and developed using DAB substrate (Quanto, Thermo Scientific, Freemont, CA). Tau mRNA and the selected oligomer ISH indicate uniform distribution of tau mRNA reduction and oligomer across the mouse brain following a single *i.c.v.* bolus injection of 100µg oligomer (data not shown).

[0344] Results Oligonucleotides show potent knockdown of Tau protein in primary hTau neurons with good tolerability *in vitro* and *in vivo* when administered directly into the

cerebral spinal fluid (CSF) via intra-cerebroventricular or intrathecal dosing (see, *e.g.*, Figure 4). They also display robust, durable tau reduction in the brain following intra-cerebroventricular administration of 100ug in C57 bl6 mice (Figure 4). Inhibition of calcium oscillations in primary neurons was not observed in primary neurons treated with these oligomers. This inhibition of calcium oscillations in primary neurons was a strong indication of acute *in vivo* tolerability issues related to network dysfunction when injected into CSF directly.

[0345] The oligomers sustained Tau reduction following a 100µg intra-cerebroventricular (ICV) bolus injection. 100µg/5µl was injected into wt C57 mice, 3 Month study in wt mice; N=12. Robust and sustained Tau RNA and protein reduction was achieved; 3x33ug intra-cerebroventricular bolus injections produced similar results (data not shown).

[0346] Dose dependent au RNA reduction was also observed following intrathecal (IT) injection of selected oligomers into lumbar ported rats (data not shown). A single bolus IT injection of 300µg of the selected gapmers was injected into lumbar catheterized rats (as described above). Robust and sustained reduction of brain Tau mRNA was observed at both 3 days and 4 weeks following the single bolus administration using the proposed clinical route of administration of these representative oligomers. IT administration is the preferred clinical route for the treatment of tau dependent disorders.

Tau protein reduction

[0347] Tau ASOs in the 3'UTR were administered at 100µg intra-cerebroventricular (ICV) to hTau or wild type Bl6 mice in order to understand the hysteresis of Tau protein reduction with respect to mRNA reduction. During these studies, many of the Tau ASOs were not tolerated beyond 4 weeks following a single 100µg ICV bolus dose. Some of the most potent Tau ASOs in this region also reduced expression of an unintended target Ras homolog gene family, member A ("RhoA"). RhoA is a small GTPase protein of Rho family. While the effects of RhoA activity are not all well known, it is primarily associated with cytoskeleton regulation, mostly actin stress fibers formation and actomyosin contractility. In humans, it is encoded by the gene *RHOA*. The *RHOA* gene contains the sequence of actttattccaaatacactcttt (SEQ ID NO: 267). Figure 5 shows that the *RHOA* gene fragment has one to four basepair mismatches with selected oligomers (*e.g.*, ASO-000757, ASO-000755, or ASO-000753).

- [0348] Certain traditional gapmer sequences were further modified in the gap design and the wing design. In particular, the traditional gapmer design was converted to an alternating gapmer design (e.g., ASO-001967, ASO-001941, ASO-001933, and ASO-1940). Figure 5 shows that the traditional gapmers are not tolerated beyond 4 weeks following a single 100µg ICV bolus dose while the alternating gapmers exhibit tolerability beyond 4 weeks.
- [0349] Figure 5 also shows that tubulin (Tuj1) was highly correlated with long term tolerability for the ASOs shown. Rho A reduction greater than 25% also correlated with lack of long term tolerability (greater than 4 weeks following a single ICV bolus injection of 100µg of each ASO shown in Figure 5).
- [0350] ASO-001933 (100 µg - 200 µg) was administered as a single bolus intracerebroventricularly (ICV) in mice, as described above, and produced greater than 50% reduction of brain Tau protein that was sustained for 4-12 weeks in hTau mice. At these dose levels, there were no clinical signs of toxicity and no gross or histologic findings observed over the 20-week period following a single ICV dose in mouse. ASO-000013 was also administered and gave results similar to ASO-001933. A single ICV bolus injection of 100µg produced no adverse changes in cognition as assessed by novel object recognition or contextual fear conditioning, motor function as assessed by catwalk, rotorod and running wheel (data not shown). In a Tau knock out mouse carrying the entire human tau gene (hTau), the EC₅₀ for reduction of human Tau brain mRNA and protein was ~2.72 µg/g (414 nM). As Figure 7 shows, ASO-001933 (Tau ASO) produces durable, dose responsive brain hTau protein reduction after a single intracerebroventricular (ICV) injection in hTau mouse brain. Saline or 50, 100, 150 and 200µg of Tau ASO was injected ICV in hTau mice (n=10 per group). The frontal cortical region was dissected eight weeks post dose to determine total Tau protein levels by ELISA (BT2/HT7). Two-way ANOVA and Bonferroni post hoc analysis were used ***p<0.001. Error bars represent SEM.

Example 8: Construction of Oligomers Targeting 5' UTR and/or Exon 2

- [0351] A number of oligomers were designed to target the 5' UTR and/or exon 2 of *MAPT* pre-mRNA. For example, the oligomers were constructed to target nucleotides 72,802-73,072 of SEQ ID NO: 1. The exemplary sequences of the oligomers are described in

Figure 6A and 6B. In some embodiments, the oligomers were designed to be gapmers or mixmers. Figures 6A and 6B show non-limiting examples of the oligomer design for selected sequences. The same methods can be applied to any other sequences disclosed herein. The gapmers were constructed to contain locked nucleic acids – LNAs (upper case letters). For example, a gapmer can have Beta-deoxy LNA at the 5' end and the 3' end and have a phosphorothioate backbone. But the LNAs can also be substituted with any other nucleotide analogs and the backbone can be other types of backbones (*e.g.*, a phosphodiester linkage, a phosphotriester linkage, a methylphosphonate linkage, a phosphoramidate linkage, or combinations thereof). A reference to a SEQ ID number includes a particular sequence, but does not include an oligomer design.

[0352] The oligomers were synthesized using methods well known in the art. Exemplary methods of preparing such oligomers are described in Barciszewski *et al.*, Chapter 10 – "Locked Nucleic Acid Aptamers" in *Nucleic Acid and Peptide Aptamers: Methods and Protocols*, vol. 535, Gunter Mayer (ed.) (2009), the entire contents of which is hereby expressly incorporated by reference herein.

Example 9: Tau mRNA and Protein Reduction in Cynomolgus monkeys

[0353] Progressive supranuclear palsy (PSP) is a neurodegenerative syndrome that is clinically characterized by progressive postural instability, supranuclear gaze palsy, parkinsonism and cognitive impairment. PSP is defined neuropathologically by the accumulation of tau-positive neurofibrillary tangles in brain regions extending from the cerebral cortex, basal ganglia to the cerebellum and brainstem. The most severely affected brain regions include the brainstem substantia nigra, pontine nuclei and the cerebellar dentate nucleus. Tauopathy in these regions is believed to underpin several clinical features of PSP such as postural instability, dysarthria and gaze palsy. Suppression of Tau mRNA transcripts and, consequently, protein in the brain regions, may have therapeutic significance for treatment of PSP patients.

[0354] Subjects were male cynomolgus monkeys weighing 3.5-10.0 kg at the start of the study. Each was implanted with an intrathecal CSF catheter entering at the L3 or L4 vertebrae extending to approximately the L1 vertebra. The proximal end of the catheter was connected to a subcutaneous access port. CSF was collected through the port by gravity flow to a maximum of 0.5 ml CSF per sample. The CSF was centrifuged and the

supernatant was kept at -90° C until analyzed. Blood plasma obtained from an available vein was kept at -90° C until analyzed.

- [0355] Cynomolgus monkeys were administered with ASO-1933, which was dissolved in saline, at 0.33 ml/min in a 1.0 ml volume followed by a 0.5 ml sterile water flush. Total infusion time was 4.5 min.
- [0356] Cynomolgus monkeys were administered the appropriate volume of a commercially available euthanasia solution while anesthetized with ketamine and isoflurane. Necropsy tissues were obtained immediately thereafter and the brain was transferred to wet ice for dissection. Areas of interest were dissected using 6mm slices in an ASI Cyno Brain Matrix as well as free handed techniques. Samples were placed fresh in RNAlater, or frozen on dry ice for later analysis. Some slices were frozen intact for immunohistochemical analysis. Slices were placed in a weigh boat and floated on isopentane cooled with dry ice. Once frozen, slices were stored at -90° C until analysis.
- [0357] For brain block sectioning, the frozen brain blocks were cut on a cryostat coronal sections, and sections were thaw-mounted onto super frost slides, dried, re-frozen on dry ice, and stored at -80° C until use. Brain sections collected from the cynomolgus monkey dosed with vehicle, ASO-1933 at 16 mg (1 x 16) or ASO-1933 at 16 mg twice (2 x 16, with 2 weeks apart) were used for the *in situ* hybridization (ISH) study.
- [0358] In order to measure Tau mRNA expression using [³⁵S]labeled antisense ISH, a Tau DNA template and [³⁵S]labeled antisense probes were synthesized. A Tau DNA template (425 bp, 687 – 1111, accession number: XM_005584540.1) was amplified from a cynomolgus monkey cDNA library (Zyagen KD-201) by PCR using forward primer 5'-CAA GCT CGC ATG GTC AGT AA-3' (SEQ ID NO: 339) and reverse primer 5'-AAT TAA CCC TCA CTA AAG GGA GA TTC TCA GTG GAG CCG ATC TT-3' (SEQ ID NO: 340). Products of desired size were observed by gel electrophoresis. The Tau DNA template was transcribed with T3 RNA polymerase (Invitrogen AM1316) using [³⁵S]UTP (Perkin Elmer NEG-739) to produce a [³⁵S]labeled antisense ISH probe.
- [0359] To measure Tau mRNA ISH using [³⁵S]labeled antisense probe, slides were thawed, fixed in 4% paraformaldehyde for 15 min at 4°C followed by rinsing. Slides were then treated in acetic anhydride/triethanolamine followed by rinsing. Slides were pre-hybridized in pre-hybridization solution at 50°C for 3 hours and hybridized with 1.5 x 10⁴ cpm/ul [³⁵S]riboprobe (0.75 ml/slide) in hybridization solution. After hybridization, slides

were washed at room temperature. Slides were then treated with Rnase A at 37°C, washed twice, followed by a high stringency wash. The sections were dehydrated in 90% alcohol containing 0.3 M NH₄Ac, dried, and exposed against phosphor screen (Perkin Elmer PPN 7001487). After exposure, autoradiographic images on the screen were captured and analyzed using Cyclone storage phosphor system and OptiQuant Acquisition and Analysis software (PerkinElmer, Waltham, MA).

[0360] QUANTIGENE[®] ViewRNA tissue ISH was used to detect Tau mRNA expression at the subnucleus and cellular levels. An antisense probe (type-1) targeting Tau mRNA (2344-3300, accession number: XM_005584529) was synthesized by Affymetrix. Slides were fixed in 4% formaldehyde in phosphate buffered saline (PBS). After passing through alcohol gradients for 10 minutes each, slides were dried, followed by protease QF digestion. Subsequently, sections were washed and hybridized with the target probe. Slides were then washed in wash buffer and stored in storage buffer overnight. Slides were then processed through a series of sequential PreAmp and Amp hybridization steps. The sections were incubated with Label Probe AP followed by incubation with Fast Red Substrate, rinsed in PBS, and counterstained using either Gill's Hematoxylin or DAPI. Slides were coverslipped using DAKO ultramount mounting medium and stored. Labeled Tau mRNA was visualized using either a Leica brightfield microscope or a Leica confocal fluorescence microscope (excitation: 630 nm; emission: 760).

[0361] To measure Tau protein expression, Tau12 (BioLegend, San Diego, CA, epitope to amino acids 6–18 on tau 441 sequence) and BT2 (Thermo Scientific, Rockville, IL, epitope to amino acids 194-198) were used to coat Costar 3925 ELISA plates at 2.5 and 1 µg/ml, respectively. Plates were incubated for 1 h at 37 °C before washing with TBS with 0.05% Tween-20 (TBST). Non-specific binding was blocked by the addition of 3% bovine serum albumin (BSA) in TBS with 0.1% Tween-20 for 4 h at room temperature with shaking. Plates were washed with TBST before the addition of samples or standard curve generated with recombinant h-tau441 protein, both of which were prepared in TBST plus 1% BSA. Plates containing standard curve and samples were incubated overnight at 4 °C with shaking. The following detection antibodies were conjugated with alkaline phosphatase (AP) using the Lightning Link Conjugation Kit (Novus Biologicals, Littleton, CO): BT2 and HT7 (Thermo Scientific, epitope of 159-163). AP-conjugated detection antibodies were diluted in TBST plus 1% BSA and co-incubated with samples

and standard curve for 1 h at room temperature with shaking. After washing with TBST, Tropix CDP-Star Ready-to-Use with Sapphire-II AP substrate (Applied Biosystems, Bedford, MA) was added for 30 min. Chemiluminescent signal was determined using a Perkin Elmer EnVision microplate reader (Waltham, MA).

[0362] The N-terminal tau sandwich ELISA (Tau12-BT2) consists of the anti-tau antibody Tau12 as capture and detection with an alkaline phosphatase (AP) conjugate of the anti-tau antibody BT2. The mid-domain tau sandwich ELISA (BT2-HT7) consists of the anti-tau antibody BT2 as the capture antibody and detection with an alkaline phosphatase (AP) conjugate of the anti-tau antibody HT7. High binding black well ELISA plates (Costar, Corning, Tewksbury, MA) were coated with anti-Tau BT2 monoclonal antibody (Thermo, Waltham, MA) at 2.5 µg/ml or Tau12 anti-tau monoclonal antibody (Covance) at 5 µg/ml in tris buffered saline (50 µL/well). The plates were washed with tris buffered saline containing 0.05% tween-20 (TBS-T) followed by blocking at room temperature with shaking in 3% BSA/TBS (BSA from Roche, Indianapolis, IN). The plates were rewashed as listed above followed by sample addition in triplicate (50 µL/well). Cynomolgus monkey CSF samples were diluted 1:30 (BT2/HT7) or 1:25 (Tau12/BT2) in 1% BSA/TBS-T. A Tau 441 (R-peptide, Bogart, GA) standard curve was made. The samples were incubated on the ELISA plate overnight at 4° C with shaking. AP conjugated HT7 or BT2 was diluted to 0.25 µg/ml (HT7) or 0.1 µg/ml (BT2) in 1% BSA/TBS-T was added to the plates (50 µL/well) for co-incubation with standards and samples for 1 hour at room temperature with shaking. The plates were re washed followed by the addition of chemiluminescent substrate (Tropix CDP Star, Applied Biosystems, Grand Island, NY) (100 µL/well) and incubation at room temperature with shaking for 30 minutes. The plates were read on a Perkin Elmer TopCount. Unknown sample values were read off the Tau-441 standard curve using GraphPad Prism software.

[0363] These studies demonstrate that intrathecally-applied Tau ASO distributes to the substantia nigra, pontine nuclei and dentate nucleus and suppresses Tau mRNA expression in these brain regions in Cynomolgus monkeys following intrathecal administration of ASO-001933 following two doses (2 week apart) of 16 mg (2 x 16). Figures 7A show *in situ* hybridization (ISH) autoradiographic images of tau mRNA expression (lighter shades) in the substantia nigra, pontine nuclei and dentate nucleus in the monkeys dosed with vehicle or ASO-001933 2 x 16 mg (1 week apart). As Figure 8B

shows, ASO-001933 produced profound suppression of Tau mRNA expression in all three regions in both monkeys. The Tau mRNA knockdown effect produced by ASO-001933 was further demonstrated using the QUANTIGENE[®] ViewRNA ISH assay (data not shown). Figure 8B shows that in the vehicle-treated monkey, a high intensity Tau mRNA labeling was present, primarily, in neuronal cell bodies in the substantia nigra, pontine nuclei and dentate nucleus. In cynomolgus monkeys, two single 16mg intrathecal doses of ASO-001933, one week apart were administered to assess anatomic distribution of Tau mRNA reduction in anatomic brain regions where pathologic Tau accumulates in PSP (Figure 8A).

[0364] In monkeys, a single intrathecal (IT) dose of 4mg of ASO-001933 produced Tau mRNA reductions between 58% to 80% in cortical brain regions and 63% in cerebellum within 2 weeks post dose (data not shown). These areas of the brain are believed to be important for treatment of Tau-dependent dysfunction in PSP (neurodegenerative tauopathies) and Dravet syndrome (epilepsy and autism spectrum disorders), leading indications for Tau antisense molecules like ASO-001933.

[0365] Consistent with Tau mRNA, Figures 9A and 9B show that the ASO-001933 administration as IT bolus injection (2 doses of 8mg given 2 weeks apart) in monkeys is capable of reducing about 70% of Tau protein in the brain (Figure 9A) about 60% in the CSF (Figure 9B). The Tau protein expression was observed 12 weeks following the ASO administration. Similarly, the ASO-001933 administration as a single ICV intracerebroventricular injection (100µg) in mice is capable of reducing about 50% of Tau protein in the brain (data not shown) and about 34% of Tau protein in the CSF (data not shown). These data suggest that reduction of CSF Tau protein may be a clinically accessible biomarker of target engagement.

[0366] ASO-002038 was administered as a single bolus intracerebroventricularly (Figure 10A: ICV at 25 - 150 µg) or intrathecally (Figure 10B: IT at 400 - 900µg) in mice or in rats, as described in Example 5. ASO-002038 produced dose dependent hTau mRNA reduction in the brain with a calculated EC₅₀ value ~ 598nM in mice. At these dose levels, there were no clinical signs of toxicity and no gross or histologic findings observed following a single ICV dose in mouse. Many ASOs including ASO-000013, ASO-001933, ASO-001967, ASO-001940, ASO-001941, and others produced similar

hTau dose dependent reduction, EC₅₀ for reduction of human Tau brain mRNA, and were well tolerated in mice (data not shown).

Example 10: Quantigene Analysis of Tau, Rho and Tubulin mRNA Expression

- [0367]** To measure tau, rhoA and tubulin mRNA reduction, primary neuronal cultures were established from the forebrain of E18 transgenic mice expressing the human tau transgene on a mouse tau knockout background. (Andorfer *et al. J Neurochem* 86:582–590 (2003)). Cultures were prepared as described in Example 2. Alternatively, iNeurons from Cellular Dynamics Inc., were used per manufacturer specifications.
- [0368]** Lysis: Cells were plated on poly-D-lysine coated 96 well plates at 50,000 cells per well and maintained in Neurobasal media containing B27, glutamax and Penicillin-Streptomycin. ASOs were diluted in water and added to cells at DIV01 to a final concentration of 5 μ M. For IC₅₀ determinations, neurons were treated with a top concentration of 5 μ M and a concentration response dilution of 1:3 was used to define the IC₅₀. Following ASO treatment, neurons were incubated at 37°C for 5 days to achieve steady state reduction of mRNA. Media was removed and cells were washed 1X in DPBS and lysed as follows. Measurement of lysate messenger RNA was performed using the Quantigene 2.0 Reagent System (Affymetrix), which quantitates RNA using a branched DNA-signal amplification method reliant on the specifically designed RNA capture probe set. The working cell lysis buffer solution was made by adding 50 μ l proteinase K to 5ml of pre-warmed Lysis mix and diluted to 1:4 final dilution with dH₂O. The working lysis buffer was added to the plate (150 μ l/ well), triturated to mix, sealed and incubated. Following lysis the wells were titrated to mix and stored at -80°C or assayed immediately.
- [0369]** Assay: Lysates were diluted in lysis mix dependent on the specific capture probe used (tau, RhoA or tubulin). 80 μ l/ well total were then added to the capture plate (96 well polystyrene plate coated with capture probes). Working probe sets reagents were generated by combining nuclease-free water 12.1 μ l, lysis mixture 6.6 μ l, blocking reagent 1 μ l, specific 2.0 probe set 0.3 μ l (human MAPT catalogue #15486, human RHOA catalogue #SA-11696, or human beta 3 tubulin catalogue #SA-15628) per manufacturer instructions (QuantiGene 2.0 Affymetrix). Then 20 μ l working probe set reagents were added to 80 μ l lysate dilution (or 80 μ l lysis mix for background samples)

on the capture plate. Plates were centrifuged and then incubated for 16-20 hours at 55°C to hybridize (target RNA capture). Signal amplification and detection of target RNA was begun by washing plates with buffer 3 times to remove unbound material. 2.0 Pre-Amplifier hybridization reagent (100 µl/well) was added, incubated at 55°C for 1 hour then aspirated and wash buffer was added and aspirated 3 times. The 2.0 Amplifier hybridization reagent was then added as described (100 µl/well), incubated for 1 hour at 55°C and the wash was repeated as described previously. The 2.0 Label Probe hybridization reagent was added next (100 µl/well), incubated for 1 hour at 50°C and the wash was repeated as described previously. Lastly, the plates were centrifuged to remove any excess wash buffer and 2.0 Substrate was added (100 µl/well). Plates were incubated for 5 minutes at room temperature and plates were imaged on a PerkinElmer Envision multilabel reader in luminometer mode within 15 minutes.

[0370] Data determination: For the gene of interest, the average assay background signal was subtracted from the average signal of each technical replicate. The background-subtracted, average signals were divided by the background subtracted average signal for the housekeeping tubulin RNA. The percent inhibition for the treated sample was calculated relative to control treated sample lysate. Results of Quantigene assays for cells treated with ASOs are shown in Figures 11A and 11B.

CLAIMS

1. An oligomer of at least 10 contiguous nucleotides in length, comprising region A, region B, and region C (A-B-C),
wherein region B comprises at least 5 consecutive nucleoside units and is flanked at 5' by region A of 1-8 contiguous nucleoside units and at 3' by region C of 1-8 contiguous nucleoside units, wherein region B, when formed in a duplex with a complementary RNA, is capable of recruiting RNaseH, and
wherein region A and region C are selected from the group consisting of:
 - (i) region A comprises a 5' LNA nucleoside unit and a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit, and, region C comprises at least two 3' LNA nucleosides; or
 - (ii) region A comprises at least one 5' LNA nucleoside and region C comprises a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units, and
 - (iii) region A comprises a 5' LNA nucleoside unit and a 3' LNA nucleoside unit, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside unit; and region C comprises a 5' LNA nucleoside unit, at least two terminal 3' LNA nucleoside units, and at least one DNA nucleoside unit between the 5' LNA nucleoside unit and the 3' LNA nucleoside units.
2. The oligomer according to claim 1, wherein region A or region C comprises 1, 2, or 3 DNA nucleoside units.
3. The oligomer according to claim 1 or 2, wherein region B comprises at least five consecutive DNA nucleoside units.
4. The oligomer according to any one of claims 1 – 3, wherein region A comprises two 5' terminal LNA nucleoside units.

- [illegible]

[illegible]

LLLDDLDDDDDDDDDDDDLLLL, LLLDLDDDDDDDDDDDDLDDL,
 LLLLDDDDDDDDDDDDLDDL, LLLLDDDDDDDDDDDDLDDL, and
 LLDDLDDDDDDDDDDDDL; wherein L represents a beta-D-oxy LNA nucleoside,
 and D represents a DNA nucleoside.

10. The oligomer according to any one of claims 1 – 6, wherein the contiguous nucleotides comprise an alternating sequence of LNA and DNA nucleoside units, 5' – 3', selected from the group consisting of : 2-3-2-8-2, 1-1-2-1-1-9-2, 3-10-1-1-2, 3-9-1-2-2, 3-8-1-3-2, 3-8-1-1-1-2, 3-1-1-9-3, 3-1-1-8-1-1-2, 4-9-1-1-2, 4-8-1-2-2, 3-3-1-8-2, 3-2-1-9-2, 3-2-2-8-2, 3-2-2-7-3, 5-7-1-2-2, 1-1-3-10-2, 1-1-3-7-1-2-2, 1-1-4-9-2, 2-1-3-9-2, 3-1-1-10-2, 3-1-1-7-1-2-2, 3-1-2-9-2, 4-7-1-3-2, 5-9-1-1-2, 4-10-1-1-2, 3-11-1-1-2, 2-1-1-10-1-1-2, 1-1-3-9-1-1-2, 3-10-1-2-2, 3-9-1-3-2, 3-8-1-1-1-2-2, 4-9-1-2-2, 4-9-1-1-3, 4-8-1-3-2, 4-8-1-2-3, 4-8-1-1-1-1-2, 4-7-1-2-1-1-2, 4-7-1-1-1-2-2, 2-1-2-11-2, 2-1-3-8-1-1-2, 3-1-1-11-2, 3-1-1-9-1-1-2, 3-1-1-8-1-2-2, 3-1-1-7-1-1-1-1-2, 4-9-2-1-2, 4-7-1-3-3, 5-9-1-1-3, 5-9-1-2-2, 4-10-2-1-2, 4-10-1-1-3, 4-10-1-2-2, 3-11-2-1-2, 3-11-1-1-3, 5-9-2-1-2, 3-11-1-2-2, 2-1-2-9-1-2-2, 3-1-1-10-1-1-2, 3-1-1-9-1-2-2, 4-9-1-1-1-1-2, 4-8-2-1-1-1-2, 1-1-3-10-2-1-2, 2-1-2-10-2-1-2, 2-1-1-12-4, 2-2-1-11-4, 3-1-1-11-4, 2-1-1-13-3, 2-1-2-11-4, 2-2-1-12-3, 3-11-1-2-3, 3-1-1-12-3, 2-1-2-12-3, 4-11-2-1-2, 4-10-2-2-2, 3-2-1-9-1-1-3, 2-2-1-1-1-9-4, 2-2-2-9-1-1-3, 3-1-1-9-1-1-1-1-2, 2-1-2-9-1-2-3, 3-1-1-10-1-1-3, 2-1-1-2-1-9-4, 4-9-1-1-1-2-2, 3-1-1-9-1-2-3, 2-1-1-1-1-10-4, 2-1-2-10-1-1-3, 2-1-1-1-1-9-2-1-2, 2-2-2-9-2-1-2, 4-9-1-2-1-1-2, 3-2-1-9-2-1-2, 2-1-2-9-2-2-2, 2-1-1-1-1-9-1-1-3, 3-1-1-9-2-2-2, 2-2-2-10-4, 2-1-2-9-1-1-1-1-2, 4-10-1-2-3, 3-2-1-10-4, 3-1-1-10-2-1-2, 4-10-1-1-1-1-2, 4-11-1-1-3, and 2-2-2-10-1-1-2; wherein the first numeral represents an number of LNA units, the next a number of DNA units, and alternating LNA and DNA regions thereafter.
11. The oligomer according to any one of claims 1-10, wherein the LNA nucleoside units present in regions A and C are beta-D LNA units, such as beta-D-oxy LNA nucleoside units or (S)cEt nucleoside LNA units.
12. The oligomer according to any one of claims 1 – 11, wherein the oligomer comprises at least one phosphorothioate internucleoside linkage.

13. A conjugate comprising the oligomer according to any one 1-12 covalently attached to at least one non-nucleotide or non-polynucleotide moiety.
14. The conjugate according to claim 13, wherein the at least one non-nucleotide or non-polynucleotide moiety is selected from the group consisting of proteins, peptides, glycoproteins, antibodies, antibody binding domains, fatty acids, sterols, sugar residues, a GalNAc conjugate such as a trivalent GalNAc cluster, polymers, PEG, and two or more combinations thereof.
15. A pharmaceutical composition comprising the oligomer of any one of claims 1 – 12, or the conjugate of claim 13 or 14, and a pharmaceutically acceptable excipient, carrier, or diluent.
16. Use of the oligomer of any one of claims 1 – 12, or the conjugate of claim 13 or 14 for the manufacture of a medicament in treating a disease or condition.
17. An *in vivo* or *in vitro* method for modulating the expression of an RNA in a cell which expresses said RNA, said method comprising contacting an effective amount of the oligomer of any one of claims 1-12, the conjugate of claim 13 or 14 or the pharmaceutical composition of claim 15 with said cell.
18. A method of producing an oligomer having a reduced non-specific binding comprising synthesizing the oligomer of any one of claims 1 to 12.

Figure 1 (cont.)

[illegible]

Figure 1 (cont.)

[illegible]

Figure 1 (cont.)

[illegible]

Figure 1 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO	primRNA start NG_07398	premRNA end NG_07398	Chemical Structure
ASO-001941-mm3	ASO-002341	TTaTTtccaaattcaCTT ~~G~~	170	138886	138905	OxyTs OxyTs DNAAs OxyTs OxyTs DNAts DNACs DNACs DNAAs DNAAc DNATs DNATs DNATs DNATs DNATs OxyGs
ASO-001941-mm4	ASO-002353	A ~~A~~ TaTTtccagaattcaCTtTT	171	138886	138905	OxyAs OxyTs DNAAs OxyTs OxyTs DNAts DNACs DNACs DNAAs DNAGs DNAAc DNATs DNATs DNATs DNATs DNATs OxyTs OxyTs
ASO-001941-mm5	ASO-002365	TTaTTtccaagttcaCTtTC	172	138886	138905	OxyTs OxyTs DNAAs OxyTs OxyTs DNAts DNACs DNACs DNAAs DNAAc DNAGs DNATs DNATs DNATs DNATs DNATs OxyTs OxyMCs
ASO-001941-mm6	ASO-002377	TTaTTtccagattctgCTtTT	173	138886	138905	OxyTs OxyTs DNAAs OxyTs OxyTs DNAts DNACs DNACs DNAAs DNAGs DNAAc DNATs DNATs DNATs DNATs DNATs OxyTs OxyTs
ASO-001942-mm1	ASO-002363	CTtTAAttccaagtacCacTT	212	138888	138907	OxyMCs OxyTs DNAts OxyTs OxyAs DNAts DNATs DNATs DNACs DNACs DNAAc DNAGs DNAAs DNATs DNATs OxyMCs OxyAs DNACs OxyTs OxyTs
ASO-001942-mm2	ASO-002375	CTtTAAttccaattCACtG	213	138888	138907	OxyMCs OxyTs DNAts OxyTs OxyAs DNAts DNATs DNATs DNACs DNACs DNAAc DNAAs DNATs DNATs OxyMCs OxyAs DNACs OxyTs OxyGs
ASO-001942-mm3	ASO-002292	CTtTAAttccaattCGcTT	214	138888	138907	OxyMCs OxyTs DNAts OxyTs OxyAs DNAts DNATs DNATs DNACs DNACs DNAAc DNAAs DNATs DNATs OxyMCs OxyGs DNACs OxyTs OxyTs
ASO-001942-mm4	ASO-002304	CTtTAAttccaggattCACtA	215	138888	138907	OxyMCs OxyTs DNAts OxyTs OxyAs DNAts DNATs DNATs DNACs DNACs DNAAc DNAGs DNAAs DNATs DNATs OxyMCs OxyAs DNACs OxyTs OxyAs
ASO-001942-mm5	ASO-002316	CTtTAAttccagggttacCacTT	216	138888	138907	OxyMCs OxyTs DNAts OxyTs OxyAs DNAts DNATs DNATs DNACs DNACs DNAAc DNAGs DNAGs DNATs DNATs OxyMCs OxyAs DNACs OxyTs OxyTs

Figure 1 (cont.)

[illegible]

Figure 1 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO	premRNA start NG_007398	premRNA end NG_007398	Chemical Structure
15874-01		acTtTtAC		4	03	DNAts DNACs DNAAs DNACs OxyTs DNAts OxyTs DNAts OxyAs OxyMCs
SPC-15876-01	ASO-002270	TTATttccaaatt caCtTTT	176	13888 6	1389 05	OxyTs OxyTs OxyAs OxyTs DNAts DNAts DNACs DNACs DNACs DNAAs DNACs OxyTs OxyTs OxyTs
SPC-15877-01	ASO-002277	TTATttccaaatt cACttTT	177	13888 6	1389 05	OxyTs OxyTs OxyAs OxyTs DNAts DNAts DNACs DNACs DNACs DNAAs DNACs DNAts OxyTs OxyTs
SPC-15878-01	ASO-002284	ATtTccAaattc acttTTAC	118	13888 4	1389 03	OxyAs OxyTs DNAts OxyTs DNACs DNACs OxyAs DNACs DNACs DNAts DNAts DNACs DNAAs DNAts
SPC-15880-01	ASO-002238	ATTtccaaattc actTtTAC	119	13888 4	1389 03	OxyAs OxyTs OxyTs OxyTs DNACs DNACs DNACs DNACs DNAts OxyTs OxyAs OxyMCs
SPC-15881-01	ASO-002247	TTaTttccaaat tcacTTTT	178	13888 6	1389 05	OxyTs OxyTs DNAAs OxyTs OxyTs DNAts DNAts DNACs DNACs DNACs DNAts OxyTs OxyTs
SPC-15883-01	ASO-002263	ATtTcCaaattc actTTtAC	120	13888 4	1389 03	OxyAs OxyTs DNAts OxyTs DNACs OxyMCs DNACs DNAAs DNACs DNAts DNAts DNACs DNAts
SPC-15884-01	ASO-002271	TTAtTtccaaatt cacTTTT	179	13888 6	1389 05	OxyTs OxyTs OxyAs DNAts OxyTs DNAts DNACs DNACs DNACs DNAts OxyTs OxyTs
SPC-15885-01	ASO-002278	TTaTtTccaaat tcACttTT	180	13888 6	1389 05	OxyTs OxyTs DNAAs OxyTs OxyTs DNAts DNAts DNACs DNACs DNACs DNAts OxyTs OxyTs
SPC-15886-01	ASO-002285	TATTtccaaatt cacTTtTA	149	13888 5	1389 04	OxyTs OxyAs OxyTs OxyTs DNAts DNACs DNACs DNACs DNAts OxyTs OxyAs
SPC-15887-01	ASO-002230	TATTtccaaatt cacTtTTA	150	13888 5	1389 04	OxyTs OxyAs OxyTs OxyTs DNAts DNACs DNACs DNACs DNAts OxyTs OxyAs
SPC-15888-01	ASO-002239	ATTtccaaattc actTTtAC	121	13888 4	1389 03	OxyAs OxyTs OxyTs OxyTs DNACs DNACs DNACs DNACs DNAts DNAts DNACs DNAts
SPC-15889-01	ASO-002248	TTaTttccaaat tcActTTT	181	13888 6	1389 05	OxyTs OxyTs DNAAs OxyTs OxyTs DNAts DNAts DNACs DNACs DNACs DNAts OxyTs OxyTs
SPC-15890-01	ASO-002256	TATTtccaaatt cAcTtTTA	151	13888 5	1389 04	OxyTs OxyAs OxyTs OxyTs DNAts DNACs DNACs DNACs DNAts DNAts DNAts OxyTs OxyAs

18/62

Figure 1 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO	premRNA start NG_007398	premRNA end NG_007398	Chemical Structure
ASO-000540-24	ASO-002121	TcCCTTaatttca ccCT	4	13605 4	1360 70	OxyTs DNacs OxyMCs OxyMCs DNats OxyTs DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-25	ASO-002133	TcCCTTaatttca ccCT	5	13605 4	1360 70	OxyTs DNacs OxyMCs OxyMCs OxyTs DNats DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-26	ASO-002145	TcCCTTaatttc AccCT	6	13605 4	1360 70	OxyTs DNacs OxyMCs OxyMCs OxyTs DNats DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-27	ASO-002157	TcCCTTaatttc accCT	7	13605 4	1360 70	OxyTs DNacs OxyMCs OxyMCs OxyTs OxyTs DNats DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-28	ASO-002169	TcCCTTaatttc accCT	8	13605 4	1360 70	OxyTs OxyMCs DNacs OxyMCs OxyTs OxyTs DNats DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-30	ASO-002193	TCCcTtaatttca ccCT	71	13605 4	1360 70	OxyTs OxyMCs OxyMCs DNacs OxyTs DNats DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-31	ASO-002110	TCCcTtaatttc AccCT	72	13605 4	1360 70	OxyTs OxyMCs OxyMCs DNacs OxyTs DNats DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-32	ASO-002122	TCCcTtaatttc accCT	73	13605 4	1360 70	OxyTs OxyMCs OxyMCs DNacs OxyTs OxyTs DNats DNAs DNAs DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs
ASO-000540-34	ASO-002146	TCCcTtaatttc CcCT	74	13605 4	1360 70	OxyTs OxyMCs OxyMCs OxyMCs DNats DNats DNats DNats DNats DNats DNats DNacs DNAs DNAs DNacs OxyMCs OxyTs

Figure 1 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO	premRNA start NG_007398	premRNA end NG_07398	Chemical Structure
ASO-000540-49	ASO-002204	CCCTtaatttca CcCtCA	15	136052	136069	OxyMCs OxyMCs OxyMCs OxyTs DNAts DNAas DNAas DNAts DNAts DNAts DNACs DNAas OxyMCs DNACs OxyMCs DNAts OxyMCs OxyAs
ASO-000540-50	ASO-002208	CCCTtaatttca ccCtCA	17	136052	136069	OxyMCs OxyMCs OxyMCs OxyTs DNAts DNAas DNAas DNAts DNAts DNAts DNACs OxyAs DNACs DNACs OxyMCs DNAts OxyMCs OxyAs
ASO-000540-51	ASO-002212	CCCTtaatttca cCctCA	18	136052	136069	OxyMCs OxyMCs OxyMCs OxyTs DNAts DNAas DNAas DNAts DNAts DNAts DNACs OxyAs DNACs OxyMCs DNAts OxyMCs OxyAs
ASO-000540-52	ASO-002216	TcCCTtaatttca cCcTC	19	136053	136070	OxyTs DNACs OxyMCs OxyMCs OxyTs DNAts DNAas DNAas DNAts DNAts DNAts DNACs DNAas DNACs OxyMCs DNACs OxyTs OxyMCs
ASO-000540-53	ASO-002220	TcCCTtaatttca cccTC	20	136053	136070	OxyTs OxyMCs DNACs OxyMCs OxyTs DNAts DNAas DNAas DNAts DNAts DNAts DNACs DNAas DNACs DNACs OxyTs OxyMCs
ASO-000540-54	ASO-002224	TcCCTTaatttc acCcTC	21	136053	136070	OxyTs OxyMCs DNACs OxyMCs OxyTs OxyTs DNAts DNAas DNAas DNAts DNAts DNAts DNACs DNAas DNACs OxyMCs DNACs OxyTs OxyMCs
ASO-000540-56	ASO-002201	TCCcttaatttca cCcTC	52	136053	136070	OxyTs OxyMCs OxyMCs DNACs DNAts DNAts DNAts DNAas DNAas DNAts DNAts DNAts DNACs DNAas DNACs OxyMCs DNACs OxyTs OxyMCs
ASO-000540-57	ASO-002205	TCCcTtaatttca cccTC	53	136053	136070	OxyTs OxyMCs OxyMCs DNACs OxyTs DNAts DNAts DNAts DNAas DNAas DNAts DNAts DNAts DNACs DNAas DNACs DNACs OxyTs OxyMCs
ASO-000540-58	ASO-002209	TCCcTtaatttca cCcTC	54	136053	136070	OxyTs OxyMCs OxyMCs DNACs OxyTs DNAts DNAts DNAts DNAas DNAas DNAts DNAts DNAts DNACs DNAas DNACs OxyMCs DNACs OxyTs OxyMCs

Figure 1 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO	premRNA start NG_007398	premRNA end NG_07398	Chemical Structure
ASO-000540-71	ASO-002199	TCCCTtaatttca cCtCA	25	13605 2	1360 70	OxyTs OxyMCs OxyMCs DNats DNats DNAAs DNAAs DNats DNats DNacs DNacs OxyMCs DNacs OxyTs OxyMCs OxyAs
ASO-000540-72	ASO-002203	TCCCTtaatttca cCctCA	26	13605 2	1360 70	OxyTs OxyMCs OxyMCs DNats DNats DNAAs DNAAs DNats DNats DNacs DNacs OxyMCs OxyMCs DNats OxyMCs
ASO-000540-73	ASO-002207	TCCCTtaatttca CcCtCA	27	13605 2	1360 70	OxyTs OxyMCs OxyMCs DNats DNats DNats DNats DNAAs DNAAs DNats DNats DNacs DNacs OxyMCs DNacs OxyMCs DNats OxyMCs
ASO-000540-74	ASO-002211	TCCCTtaatttc ACcCtCA	28	13605 2	1360 70	OxyTs OxyMCs OxyMCs OxyMCs DNats DNats DNats DNats DNAAs DNAAs DNats DNats DNacs DNacs OxyMCs DNacs OxyMCs DNats OxyMCs
ASO-000540-76	ASO-002219	TCCCTtaatttc acCctCA	29	13605 2	1360 70	OxyTs OxyMCs OxyMCs OxyMCs OxyTs DNats DNats DNAAs DNAAs DNats DNats DNacs DNacs DNacs OxyMCs OxyMCs DNats OxyMCs
ASO-002640	ASO-002640	CCTTaATTcac ccTCA	242	13605 2	1360 68	OxyMCs OxyMC OxyT DNat DNAAs OxyAs OxyTs DNats DNats DNats DNats DNacs DNacs DNac DNac OxyT OxyMCs OxyA
ASO-002632	ASO-002632	CCTTaATTcac ccTCA	243	13605 2	1360 68	OxyMCs OxyMC OxyT DNat DNAAs OxyAs OxyTs DNats DNats DNats DNats DNacs DNacs DNac OxyT OxyMCs OxyA
ASO-002647	ASO-002647	CCTTaATTcac ccTCA	244	13605 2	1360 68	OxyMCs OxyMC OxyT DNat DNAa OxyAs OxyTs DNats DNats DNats DNats DNacs DNacs DNac OxyT OxyMCs OxyA
ASO-002666	ASO-002666	AtTTCaatttc actTTtAC	245	13888 4	1389 03	OxyAs DNat OxyT OxyT OxyMCs DNacs DNacs DNAAs DNAAs DNats DNats DNats DNacs DNacs DNats OxyT OxyT DNAT OxyAs OxyMC
ASO-002659	ASO-002659	AtTTCaatttc actTTtAC	246	13888 4	1389 03	OxyAs DNat OxyT OxyT OxyMCs DNacs DNacs DNAAs DNAAs DNats DNats DNats DNacs DNacs DNats OxyTs OxyT DNAT OxyAs OxyMC
ASO-002652	ASO-002652	AtTTCCaaatttc actTTtAC	247	13888 4	1389 03	OxyAs OxyT DNAT DNAT OxyMC OxyMCs DNacs DNacs DNAAs DNAAs DNats DNats DNats DNats DNats DNats OxyTs DNAT OxyT OxyAs OxyMC

Figure 1 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO	premRNA start NG_007398	premRNA end NG_007398	Chemical Structure
002399		actTTtAC		4	03	DNAts DNACs DNAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-002482	ASO-002482	ATtTcCaAattc	262	13888	1389	OxyAs OxyTs DNAts OxyTs DNACs OxyMCs DNAAs OxyAs DNAAs DNAts
ASO-002437	ASO-002437	ATtTcCaaattc	263	13888	1389	DNAts DNACs DNAAs DNACs DNAts OxyTs DNAts OxyTs OxyAs OxyMC
ASO-002425	ASO-002425	ATtTcCAaattc	264	13888	1389	OxyAs OxyTs DNAts OxyTs DNACs OxyMCs OxyAs DNAAs DNAts
		acTTtTAC		4	03	DNAts DNACs DNAAs DNACs OxyTs DNAts OxyTs OxyAs OxyMC

ASO number indicates unique designation for each oligomer.

ASO sequence corresponds to the oligomer sequence, left corresponds to the 5'end and right corresponds to the 3'end.

SEQ ID Number corresponds to the sequence of the oligomer listed in the sequence listing.

Figure 2

SEQ ID NO.	Oligomer Name	ASO Number	ASO Sequence	premRNA start NG_007398	premRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
2	ASO-000540-22	ASO-002192	CCCTAatttcacCctCA	136052	136068	3891	
3	ASO-000540-23	ASO-002109	CCCTAatttcacCctCA	136052	136068	3891	
4	ASO-000540-24	ASO-002121	TcCctTaatttcaccCT	136054	136070	3893	
5	ASO-000540-25	ASO-002133	TcCCTtaatttcaccCT	136054	136070	3893	
6	ASO-000540-26	ASO-002145	TcCCTtaatttcAccCT	136054	136070	3893	
7	ASO-000540-27	ASO-002157	TcCCTTaatttcaccCT	136054	136070	3893	
8	ASO-000540-28	ASO-002169	TcCCTTaatttcaccCT	136054	136070	3893	
9	ASO-000540-3	ASO-002154	CCCttaaatttcacCcTC	136053	136069	3892	
10	ASO-000540-43	ASO-002159	CCCTTaatttcaccCtCA	136052	136069	3891	
11	ASO-000540-45	ASO-002183	CCCTTaatttcacCctCA	136052	136069	3891	
12	ASO-000540-46	ASO-002195	CCCTTaatttcacCcTCA	136052	136069	3891	
13	ASO-000540-47	ASO-002196	CCCTTaatttcacCcctCA	136052	136069	3891	
14	ASO-000540-48	ASO-002200	CCCTTaatttcacCcTCA	136052	136069	3891	
15	ASO-000540-49	ASO-002204	CCCTTaatttcacCcCtCA	136052	136069	3891	
16	ASO-000540-5	ASO-002178	CCCTtaatttcAcccTC	136053	136069	3891	
17	ASO-000540-50	ASO-002208	CCCTtaatttcAccCtCA	136052	136069	3891	
18	ASO-000540-51	ASO-002212	CCCTtaatttcAcCctCA	136052	136069	3891	
19	ASO-000540-52	ASO-002216	TcCCTtaatttcacCcTC	136053	136070	3892	
20	ASO-000540-53	ASO-002220	TcCCTtaatttcacccTC	136053	136070	3892	
21	ASO-000540-54	ASO-002224	TcCCTTaatttcacCcTC	136053	136070	3892	
22	ASO-000540-55	ASO-002197	TCCcttaatttcaccCTC	136053	136070	3892	
23	ASO-000540-69	ASO-002222	TCCcTtaatttcacCctCA	136052	136070	3891	
24	ASO-000540-70	ASO-002226	TCCCTtaatttcaccCTCA	136052	136070	3891	
25	ASO-000540-71	ASO-002199	TCCCTtaatttcacCcTCA	136052	136070	3891	
26	ASO-000540-72	ASO-002203	TCCCTtaatttcacCCtCA	136052	136070	3891	
27	ASO-000540-73	ASO-002207	TCCCTtaatttcacCcCtCA	136052	136070	3891	
28	ASO-000540-74	ASO-002211	TCCCTtaatttcACcCtCA	136052	136070	3891	

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	premRNA start NG_007398	premRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
29	ASO-000540-76	ASO-002219	TCCCTtaatttcacCctCA	136052	136070	3891	
30	ASO-000540-8	ASO-002119	CCCTTaatttcaccCTC	136053	136069	3892	
31	ASO-000540-9	ASO-002131	CCCTTaatttcacCcTC	136053	136069	3892	
32	TBD-mm10	ASO-002382	CCttgATttcgccctCA	136053	136069	3891	
33	TBD-mm11	ASO-002299	CCttgATttcacccctCT	136052	136068	3891	
34	TBD-mm12	ASO-002311	CCttaGttcacccctCG	136054	136070	3891	
35	TBD-mm31	ASO-002349	TCcCTtgatttcacCctCA	136053	136071	3891	
36	TBD-mm32	ASO-002361	TCcCTtaatttcacCctCG	136052	136070	3891	
37	TBD-mm33	ASO-002373	ACcCTtaatttcacCctCA	136053	136071	3891	
38	TBD-mm34	ASO-002385	TCcCTtgatttcgCctCA	136052	136070	3891	
39	TBD-mm35	ASO-002302	TCcCTtagtttcacCctCG	136054	136072	3891	
40	TBD-mm36	ASO-002314	ACcCTtgatttcacCctCA	136054	136072	3891	
41	TBD-mm7	ASO-002346	CCttgATttcacccctCA	136053	136069	3891	
42	TBD-mm8	ASO-002358	CCttaGttcacccctCA	136053	136069	3891	
43	TBD-mm9	ASO-002370	CCttaATttcacccctCG	136052	136068	3891	
44	ASO-000540-11	ASO-002155	CCCTtaatttcacCcTC	136053	136069	3892	
45	ASO-000540-13	ASO-002179	CCCTtaatttcacCcTC	136053	136069	3892	
46	ASO-000540-14	ASO-002191	CCttaATttcacccctCA	136052	136068	3891	
47	ASO-000540-16	ASO-002120	CCTTaaTttcacccctCA	136052	136068	3891	
48	ASO-000540-17	ASO-002132	CCTTaAttcacccctCA	136052	136068	3891	
49	ASO-000540-18	ASO-002144	CCTTaATttcacccctCA	136052	136068	3891	
50	ASO-000540-19	ASO-002156	CCTTaATttcacccctCA	136052	136068	3891	

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	preRNA start NG_007398	preRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
51	ASO-000540-20	ASO-002168	CCTTaatttcacCtCA	136052	136068	3891	
52	ASO-000540-56	ASO-002201	TCCcttaatttcacCcTC	136053	136070	3892	
53	ASO-000540-57	ASO-002205	TCCcTtaatttcacccTC	136053	136070	3892	
54	ASO-000540-58	ASO-002209	TCCcTtaatttcacCcTC	136053	136070	3892	
55	ASO-000540-59	ASO-002213	TCCcTtaatttcacCcTC	136053	136070	3892	
56	ASO-000540-6	ASO-002190	CCCttaaatttcAcCcTC	136053	136069	3892	
57	ASO-000540-60	ASO-002217	TCCcTtaatttcAcCcTC	136053	136070	3892	
58	ASO-000540-61	ASO-002221	TCCcTtaatttcacCCTC	136053	136070	3892	
59	ASO-000540-62	ASO-002225	TCCcTtaatttcacCCTC	136053	136070	3892	
60	ASO-000540-63	ASO-002198	TCCcTtaatttcacCcTC	136053	136070	3892	
61	ASO-000540-64	ASO-002202	TCCcTtaatttcAcCcTC	136053	136070	3892	
62	ASO-000540-65	ASO-002206	TCCcTtaatttcacCcTC	136053	136070	3892	
63	ASO-000540-66	ASO-002210	TCCcTtaatttcacCcTC	136052	136070	3891	
64	ASO-000540-68	ASO-002218	TCCcTtaatttcacCcTC	136052	136070	3891	
65	TBD-mm25	ASO-002372	TcCCCTtgatttcacCcTC	136052	136069	3892	
66	TBD-mm26	ASO-002384	TcCCCTtaatttcgCcTC	136052	136069	3892	
67	TBD-mm27	ASO-002301	TcCCCTtagatttcacCcTC	136052	136069	3892	
68	TBD-mm28	ASO-002313	TcCCCTtgatttcgCcTC	136054	136071	3892	
69	TBD-mm29	ASO-002325	TcCCCTtagatttcgCcTC	136052	136069	3892	
70	TBD-mm30	ASO-002337	<u>A</u> cCCCTtgatttcacCcTC	136053	136070	3892	
71	ASO-000540-30	ASO-002193	TCCcTtaatttcacccCT	136054	136070	3893	
72	ASO-000540-31	ASO-002110	TCCcTtaatttcAccCT	136054	136070	3893	
73	ASO-000540-32	ASO-002122	TCCcTtaatttcacccCT	136054	136070	3893	
74	ASO-000540-34	ASO-002146	TCCcTtaatttcacCcCT	136054	136070	3893	
75	ASO-000540-39	ASO-002111	CCCttaaatttcacCcTC	136052	136069	3891	
76	ASO-000540-4	ASO-002166	CCCttaaatttcacCcTC	136053	136069	3892	

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	pre-mRNA start NG_007398	pre-mRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
77	ASO-000540-40	ASO-002123	CCCTtaattcaCctCA	136052	136069	3891	
78	ASO-000540-41	ASO-002135	CCCTtaattcAcCctCA	136052	136069	3891	
79	TBD-mm13	ASO-002323	TcCCTTgattcaccCT	136053	136069	3893	
80	TBD-mm14	ASO-002335	TcCCTTaatttcaccCA	136052	136068	3893	
81	TBD-mm15	ASO-002347	TcCCTTaatttcgcccCT	136053	136069	3893	
82	TBD-mm16	ASO-002359	TcCCTTgatttcaccCA	136053	136069	3893	
83	TBD-mm17	ASO-002371	TcCCTTgatttcaccCG	136052	136068	3893	
84	TBD-mm18	ASO-002383	TcCCTTgatttcgcccCT	136052	136068	3893	
85	ASO-002090	ASO-002090	ATTtCcaattcactTtTAC	138884	138903	6722	0
86	ASO-002043	ASO-002043	ATTtCcaattcactTTAC	138884	138903	6722	0
87	ASO-002076	ASO-002076	ATTtCcaattcacTTtTAC	138884	138903	6722	100
88	ASO-002062	ASO-002062	ATTtCcaattcactTTtTAC	138884	138903	6722	0
89	ASO-002082	ASO-002082	ATTtCcaattcactTtTAC	138884	138903	6722	0
90	ASO-001940	ASO-001940	ATTtCcaattcactTTtTAC	138884	138903	6722	100
91	ASO-001933	ASO-001933	ATTtCcaattcactTTtTAC	138884	138903	6722	100
92	ASO-002094	ASO-002094	ATTtCcaattcacTtTtTAC	138884	138903	6722	99
93	ASO-002034	ASO-002034	ATTtCcaattcactTtTAC	138884	138903	6722	0
94	ASO-002036	ASO-002036	ATTtCcaattcactTTTAC	138884	138903	6722	0
95	ASO-002084	ASO-002084	ATTtCcaattcacTTtTAC	138884	138903	6722	0
96	ASO-002037	ASO-002037	ATTtCcaattcaCtTtTAC	138884	138903	6722	0
97	ASO-002058	ASO-002058	ATTtCcaattcacTtTtTAC	138884	138903	6722	99
98	ASO-002057	ASO-002057	ATTtCcaattcaCtTtTAC	138884	138903	6722	0
99	ASO-002092	ASO-002092	ATTtCcaattcactTtTAC	138884	138903	6722	0
100	ASO-002023	ASO-002023	ATTtCcaattcacTTtTAC	138884	138903	6722	99
101	ASO-002065	ASO-002065	ATTtCcaattcactTTtTAC	138884	138903	6722	99

29/62

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	pre-mRNA start NG_007398	pre-mRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
102	ASO-002038	ASO-002038	ATTtCcaattcacTtTtAC	138884	138903	6722	99
103	ASO-002039	ASO-002039	ATtTcCaaattcacTtTtAC	138884	138903	6722	98
104	ASO-001933-mm1	ASO-002291	GtTTCcaattcacTtTtAC	138884	138903	6722	
105	ASO-001933-mm2	ASO-002303	AtTTCcagattcacTtTtAC	138884	138903	6722	
106	ASO-001933-mm3	ASO-002315	TtTTCcaattcacTtTtAC	138884	138903	6722	
107	ASO-001933-mm4	ASO-002327	GtTTCcagattcacTtTtAC	138884	138903	6722	
108	ASO-001933-mm5	ASO-002339	AtTTCcagattcacTtTtGC	138884	138903	6722	
109	ASO-001933-mm6	ASO-002351	AtTTCcagattcacTtTtAC	138884	138903	6722	
110	SPC-15857-01	ASO-002326	ATtTcCaaattcacTtTtAC	138884	138903	6722	
111	SPC-15858-01	ASO-002338	ATtTcCaaattcacTtTtAC	138884	138903	6722	
112	SPC-15860-01	ASO-002362	ATtTcCaaattcacTtTtAC	138884	138903	6722	
113	SPC-15864-01	ASO-002236	ATtTcCaaattcacTtTtAC	138884	138903	6722	
114	SPC-15868-01	ASO-002269	ATtTcCaaattcacTtTtAC	138884	138903	6722	
115	SPC-15872-01	ASO-002237	ATtTcCaaattcacTtTtAC	138884	138903	6722	
116	SPC-15873-01	ASO-002246	ATtTcCaaattcacTtTtAC	138884	138903	6722	
117	SPC-15874-01	ASO-002254	ATtTcCaaattcacTtTtAC	138884	138903	6722	
118	SPC-15878-01	ASO-002284	ATtTcCaaattcacTtTtAC	138884	138903	6722	
119	SPC-15880-01	ASO-002238	ATtTcCaaattcacTtTtAC	138884	138903	6722	
120	SPC-15883-01	ASO-002263	ATtTcCaaattcacTtTtAC	138884	138903	6722	
121	SPC-15888-01	ASO-002239	ATtTcCaaattcacTtTtAC	138884	138903	6722	
122	ASO-002055	ASO-002055	TAtTtCcaattcacTtTtTA	138885	138904	6723	99
123	ASO-002035	ASO-002035	TAtTtCcaattcacTtTtTA	138885	138904	6723	0
124	ASO-002048	ASO-002048	TAtTtCcaattcacTtTtTA	138885	138904	6723	99
125	ASO-002053	ASO-002053	TAtTtCcaattcacTtTtTA	138885	138904	6723	0
126	ASO-002067	ASO-002067	TAtTtCcaattcacTtTtTA	138885	138904	6723	100
127	ASO-002081	ASO-002081	TAtTtCcaattcacTtTtTA	138885	138904	6723	0

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	pre-mRNA start NG_007398	pre-mRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
128	ASO-001966	ASO-001966	TATTTccaaattcacTTtTA	138885	138904	6723	100
129	ASO-002025	ASO-002025	TAttTcCaaattcacTTtTA	138885	138904	6723	0
130	ASO-002033	ASO-002033	TAttTcCaaattcacTTtTA	138885	138904	6723	0
131	ASO-001960	ASO-001960	TaTTTccaaattcacTTtTA	138885	138904	6723	100
132	ASO-002056	ASO-002056	TAttTcCaaattcacTTtTA	138885	138904	6723	0
133	ASO-002063	ASO-002063	TATtTcCaaattcacTTtTA	138885	138904	6723	0
134	ASO-002089	ASO-002089	TATTtccaaattcaCttTTA	138885	138904	6723	99
135	ASO-002073	ASO-002073	TAttTcCaaattcacTTtTA	138885	138904	6723	99
136	ASO-002027	ASO-002027	TATtTccaaattcaCtTtTA	138885	138904	6723	99
137	ASO-002075	ASO-002075	TATtTccaaattcaCtTtTA	138885	138904	6723	99
138	ASO-002028	ASO-002028	TATtTccaaattcaCttTTA	138885	138904	6723	98
139	ASO-002085	ASO-002085	TAttTcCaaattcaCtTtTA	138885	138904	6723	98
140	ASO-002083	ASO-002083	TAttTcCaaattcaCtTtTA	138885	138904	6723	98
141	SPC-15859-01	ASO-002350	TAttTcCaaattcacTTtTA	138885	138904	6723	
142	SPC-15861-01	ASO-002374	TAttTcCaaattcacTTtTA	138885	138904	6723	
143	SPC-15862-01	ASO-002386	TATTtccaaattcaCtTtTA	138885	138904	6723	
144	SPC-15863-01	ASO-002227	TAttTcCaaattcacTTtTA	138885	138904	6723	
145	SPC-15865-01	ASO-002245	TAttTcCaaattcacTTtTA	138885	138904	6723	
146	SPC-15867-01	ASO-002261	TAttTcCaaattcacTTtTA	138885	138904	6723	
147	SPC-15869-01	ASO-002276	TATtTcCaaattcacTTtTA	138885	138904	6723	
148	SPC-15871-01	ASO-002228	TATTtccaaattcaCtTtTA	138885	138904	6723	
149	SPC-15886-01	ASO-002285	TATTtccaaattcacTTtTA	138885	138904	6723	
150	SPC-15887-01	ASO-002230	TATTtccaaattcacTTtTA	138885	138904	6723	
151	SPC-15890-01	ASO-002256	TATTtccaaattcaCtTtTA	138885	138904	6723	
152	SPC-15893-01	ASO-002279	TATTtccaaattcaCtTtTA	138885	138904	6723	
153	ASO-002072	ASO-002072	TTAttTcCaaattcaCtTTT	138886	138905	6724	99
154	ASO-002071	ASO-002071	TTaTtTcCaaattcacTTTT	138886	138905	6724	0
155	ASO-002080	ASO-002080	TTatTTcCaaattcacTTTT	138886	138905	6724	0

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	preRNA start NG_007398	preRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
156	ASO-001941	ASO-001941	TTaTTccaaattcaCTtTT	138886	138905	6724	100
157	ASO-002045	ASO-002045	TTaTtTccaaattcacTTT	138886	138905	6724	0
158	ASO-001934	ASO-001934	TtATTccaaattcaCTtTT	138886	138905	6724	100
159	ASO-002074	ASO-002074	TTaTtTccaaattcacTtTT	138886	138905	6724	0
160	ASO-002093	ASO-002093	TTaTtTccaaattcACtTT	138886	138905	6724	0
161	ASO-002054	ASO-002054	TTaTtTccaaattcaCTtTT	138886	138905	6724	0
162	ASO-002091	ASO-002091	TTaTtTccaaattcaCTtTT	138886	138905	6724	99
163	ASO-002064	ASO-002064	TTaTtTccaaattcaCTtTT	138886	138905	6724	99
164	ASO-002066	ASO-002066	TTaTtTccaaattCacTtTT	138886	138905	6724	99
165	ASO-002044	ASO-002044	TTaTtTccaaattcaCTtTT	138886	138905	6724	99
166	ASO-002047	ASO-002047	TTaTtTccaaattCaCtTT	138886	138905	6724	99
167	ASO-002046	ASO-002046	TTaTtTccaaattcacTTT	138886	138905	6724	97
168	ASO-001941-mm1	ASO-002317	<u>A</u> TaTtTccaaattcaCTtTT	138886	138905	6724	
169	ASO-001941-mm2	ASO-002329	TTaTtTccaaattcaCTtT <u>A</u>	138886	138905	6724	
170	ASO-001941-mm3	ASO-002341	TTaTtTccaaattcaCTtT <u>G</u>	138886	138905	6724	
171	ASO-001941-mm4	ASO-002353	<u>A</u> TaTtTccagattcaCTtTT	138886	138905	6724	
172	ASO-001941-mm5	ASO-002365	TTaTtTccaa <u>g</u> ttcaCTtT <u>C</u>	138886	138905	6724	
173	ASO-001941-mm6	ASO-002377	TTaTtTccagattc <u>g</u> CTtTT	138886	138905	6724	
174	SPC-15866-01	ASO-002253	TTaTtTccaaattcaCTtTT	138886	138905	6724	
175	SPC-15870-01	ASO-002283	TTaTtTccaaattcacTTT	138886	138905	6724	
176	SPC-15876-01	ASO-002270	TTaTtTccaaattcaCTtTT	138886	138905	6724	
177	SPC-15877-01	ASO-002277	TTaTtTccaaattcACtTT	138886	138905	6724	
178	SPC-15881-01	ASO-002247	TTaTtTccaaattcacTTT	138886	138905	6724	
179	SPC-15884-01	ASO-002271	TTaTtTccaaattcacTTT	138886	138905	6724	
180	SPC-15885-01	ASO-002278	TTaTtTccaaattcACtTT	138886	138905	6724	
181	SPC-15889-01	ASO-002248	TTaTtTccaaattcActTT	138886	138905	6724	

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	pre-mRNA start NG_007398	pre-mRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
182	SPC-15891-01	ASO-002264	TTATtccaaattcAcTTT	138886	138905	6724	
183	SPC-15892-01	ASO-002272	TTaTTtccaaattcAcTTT	138886	138905	6724	
184	SPC-15894-01	ASO-002286	TTATtccaaattcAcTTT	138886	138905	6724	
185	SPC-15895-01	ASO-002231	TTATtccaaattcAcTTT	138886	138905	6724	
186	SPC-15896-01	ASO-002240	TTATtccaaattcAcTTT	138886	138905	6724	
187	ASO-002020	ASO-002020	AcTTTatttccaaattcactTTTaC	138884	138908	6722	99
188	ASO-001967	ASO-001967	TTATtccaaattcAcTTT	138887	138906	6725	100
189	ASO-002086	ASO-002086	AcTTtatttccaaattcactTTTaC	138884	138908	6722	98
190	ASO-002029	ASO-002029	AcTTtatttccaaattcactTTTaC	138884	138908	6722	98
191	ASO-001961	ASO-001961	TtTATtccaaattcAcTTT	138887	138906	6725	99
192	ASO-002095	ASO-002095	AcTTtatttccaaattcactTTTaC	138884	138908	6722	94
193	ASO-002021	ASO-002021	AcTTtatttccaaattcactttTAC	138884	138908	6722	90
194	ASO-002068	ASO-002068	AcTTtatttccaaattcactTTTAC	138884	138908	6722	86
195	ASO-001967-mm1	ASO-002294	A TTATtccaaattcAcTTT	138887	138906	6725	
196	ASO-001967-mm2	ASO-002306	TTTATtccaa g ttcAcTTT	138887	138906	6725	
197	ASO-001967-mm3	ASO-002318	G TTATtccaaattcAcTTT	138887	138906	6725	
198	ASO-001967-mm4	ASO-002330	A TTATtccaa g attcAcTTT	138887	138906	6725	
199	ASO-001967-mm5	ASO-002342	TTTATtccaa g ttcAcTTT	138887	138906	6725	
200	ASO-001967-mm6	ASO-002354	G TTATtccaa g ttcAcTTT	138887	138906	6725	
201	ASO-002006	ASO-002006	CTTtATTtccaaattcAcTTT	138888	138907	6726	99
202	ASO-002017	ASO-002017	CTTtATTtccaaattcAcTTT	138888	138907	6726	99
203	ASO-001942	ASO-001942	CTTtATTtccaaattcAcTTT	138888	138907	6726	100
204	ASO-001935	ASO-001935	CtTTATTtccaaattcAcTTT	138888	138907	6726	100
205	ASO-002002	ASO-002002	CTTtATTtccaaattcAcTTT	138888	138907	6726	100
206	ASO-002010	ASO-002010	CTTtatttccaaattTcaCTT	138888	138907	6726	98
207	ASO-002005	ASO-002005	CtTtATTtccaaattcAcTTT	138888	138907	6726	99

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	preRNA start NG_007398	preRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
208	ASO-001998	ASO-001998	CTTAttccaaattcACTT	138888	138907	6726	99
209	ASO-001994	ASO-001994	CTTAttccaaattcACTT	138888	138907	6726	95
210	ASO-002013	ASO-002013	CTTAttccaaattcACTT	138888	138907	6726	95
211	ASO-002009	ASO-002009	CTTAttccaaattcACTT	138888	138907	6726	90
212	ASO-001942-mm1	ASO-002363	CTTAttccagattCACCTT	138888	138907	6726	
213	ASO-001942-mm2	ASO-002375	CTTAttccaaattCACCTG	138888	138907	6726	
214	ASO-001942-mm3	ASO-002292	CTTAttccaaattCGCTT	138888	138907	6726	
215	ASO-001942-mm4	ASO-002304	CTTAttccagattCACCTA	138888	138907	6726	
216	ASO-001942-mm5	ASO-002316	CTTAttccagattCACCTT	138888	138907	6726	
217	ASO-001942-mm6	ASO-002328	CTTAttccagattCACCTT	138888	138907	6726	
218	ASO-002004	ASO-002004	CTTAttccaaatTcaCT	138889	138907	6727	99
219	ASO-002012	ASO-002012	CTTAttccaaatTcaCT	138889	138907	6727	99
220	ASO-001987	ASO-001987	CTTAttccaaatTcaCT	138889	138907	6727	100
221	ASO-001992	ASO-001992	CTTAttccaaatTcaCT	138889	138907	6727	100
222	ASO-002000	ASO-002000	CTTAttccaaatTcaCT	138889	138907	6727	100
223	ASO-001996	ASO-001996	CTTAttccaaatTcaCT	138889	138907	6727	99
224	ASO-002008	ASO-002008	CTTAttccaaatTcaCT	138889	138907	6727	99
225	ASO-002015	ASO-002015	CTTAttccaaatTcaCT	138889	138907	6727	98
226	ASO-002016	ASO-002016	CTTAttccaaatTcaCT	138889	138907	6727	96
227	ASO-002011	ASO-002011	CTTAttccaaatTcaCT	138890	138907	6728	0
228	ASO-001988	ASO-001988	CTTAttccaaatTcaCT	138890	138907	6728	99
229	ASO-001993	ASO-001993	CTTAttccaaatTcaCT	138890	138907	6728	99
230	ASO-001997	ASO-001997	CTTAttccaaatTcaCT	138890	138907	6728	99
231	ASO-001997-mm1	ASO-002320	CTTAttccagattTcaCT	138890	138907	6728	
232	ASO-001997-mm2	ASO-002332	CTTAttccgaattTcaCT	138890	138907	6728	

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	premRNA start NG_007398	premRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
233	ASO-001997-mm3	ASO-002344	CTTt ~~g~~ tttccaaatTcAC	138890	138907	6728	
234	ASO-001997-mm4	ASO-002356	CTTt ~~g~~ tttccagatTcAC	138890	138907	6728	
235	ASO-001997-mm5	ASO-002368	CTTtatttccag ~~g~~ atTcAC	138890	138907	6728	
236	ASO-001997-mm6	ASO-002380	CTTt ~~g~~ tttccaa ~~g~~ tTcAC	138890	138907	6728	
237	ASO-002022	ASO-002022	TTATttccaaattcaCTtTT	138886	138905	6724	0
238	ASO-002026	ASO-002026	TTatTTccaaattcaCTTT	138886	138905	6724	99
239	ASO-002024	ASO-002024	TTAttTccaaattcaCTTT	138886	138905	6724	99
240	ASO-002049	ASO-002049	ACTTtatttccaaattcactTTtAC	138884	138908	6722	99
241	ASO-002019	ASO-002019	ActtTatttccaaattcactTTtAC	138884	138909	6722	97
242	ASO-002640	ASO-002640	CCTTaATTtcccccTCA	136052	136068	3891	34
243	ASO-002632	ASO-002632	CCTTaATTtcccccTCA	136052	136068	3891	65
244	ASO-002647	ASO-002647	CCTTaATTtcccccTCA	136052	136068	3891	34
245	ASO-002666	ASO-002666	AtTTCcaattcactTTtAC	138884	138903	6722	70
246	ASO-002659	ASO-002659	AtTTCcaattcactTTtAC	138884	138903	6722	77
247	ASO-002652	ASO-002652	AtTtCCaaattcactTtTAC	138884	138903	6722	72
248	ASO-002645	ASO-002645	AtTtCCaaattcactTtTAC	138884	138903	6722	75
249	ASO-002638	ASO-002638	AtTtCCaaattcactTtTAC	138884	138903	6722	79
250	ASO-003270	ASO-003270	AtTTCcaattcactTTtAC	138884	138903	6722	85
251	ASO-003269	ASO-003269	AtTTCcaattcactTTtAC	138884	138903	6722	61
252	ASO-003268	ASO-003268	AtTTCcaattcactTTtAC	138884	138903	6722	73
253	ASO-002673	ASO-002673	AtTTCcaattcactTTtAC	138884	138903	6722	65
254	ASO-002661	ASO-002661	TTtATTtccaaattcactTT	138887	138906	6725	42
255	ASO-002654	ASO-002654	TTtATTtccaaattcactTT	138887	138906	6725	42
256	ASO-002668	ASO-002668	TTtATTtccaaattcactTT	138887	138906	6725	28
257	ASO-002658	ASO-002658	CCTTaATTtcccccTCA	136052	136068	3891	99
258	ASO-002629	ASO-002629	AtTTCcaattcactTTtAC	138884	138903	6722	99
259	ASO-002621	ASO-002621	AtTtCCaaattcactTtTAC	138884	138903	6722	100

Figure 2 (cont.)

SEQ ID NO:	Oligomer Name	ASO Number	ASO Sequence	premRNA start NG_007398	premRNA end NG_007398	mRNA start NM_016835	Tau/Tubulin % inhibition
260	ASO-002665	ASO-002665	TTtATttccaaattcACtTT	138887	138906	6725	99
261	ASO-002399	ASO-002399	AtTTCcAaAttcactTTtAC	138884	138903	6722	66
262	ASO-002482	ASO-002482	ATtTcCaAattcactTtTAC	138884	138903	6722	66
263	ASO-002437	ASO-002437	ATtTcCaaattcacTTtTAC	138884	138903	6722	66
264	ASO-002425	ASO-002425	ATtTcCAaattcacTTtTAC	138884	138903	6722	66

ASO sequence corresponds to the oligomer sequence, left corresponds to the 5'end and right corresponds to the 3'end.

SEQ ID Number corresponds to the sequence of the oligomer listed in the sequence listing.

"mm" indicates "mismatches."

Figure 3

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
ASO-002082	ASO-002082	ATTtCcaattcactTtTAC	89	138884	138903	>100%	0.01
ASO-001988	ASO-001988	CTTTAttccaaatTcAC	228	138890	138907	>100%	0.02
ASO-002065	ASO-002065	ATtCCaaattcactTtTAC	101	138884	138903	>100%	0.02
ASO-002092	ASO-002092	ATtCCaaattcactTtTAC	99	138884	138903	>100%	0.02
ASO-002000	ASO-002000	CTTTatttccaaatTcACT	222	138889	138907	>100%	0.02
ASO-001987	ASO-001987	CTTTAttccaaatTcACT	220	138889	138907	>100%	0.02
ASO-002015	ASO-002015	CTTTAttccaaatTcACT	225	138889	138907	>100%	0.02
ASO-001989	ASO-001989	CTTTatttccaaatTcACT	266	138888	138907	0%	0.02
ASO-002034	ASO-002034	ATTtCcaattcactTtTAC	93	138884	138903	>100%	0.02
ASO-002036	ASO-002036	ATtCCaaattcactTtTAC	94	138884	138903	>100%	0.02
ASO-002006	ASO-002006	CTtTAttccaaatTcACT	201	138888	138907	0%	0.02
ASO-002043	ASO-002043	ATTtCcaattcactTtTAC	86	138884	138903	>100%	0.02
ASO-002062	ASO-002062	ATTtCcaattcactTtTAC	88	138884	138903	>100%	0.02
ASO-002023	ASO-002023	ATTtCcaattcactTtTAC	100	138884	138903	>100%	0.02
ASO-002038	ASO-002038	ATTtCcaattcactTtTAC	102	138884	138903	>100%	0.02
ASO-001992	ASO-001992	CTTTAttccaaatTcACT	221	138889	138907	>100%	0.03
ASO-001940	ASO-001940	ATTtCcaattcactTtTAC	90	138884	138903	85%	0.03
ASO-002058	ASO-002058	ATTtCcaattcactTtTAC	97	138884	138903	>100%	0.03
ASO-001935	ASO-001935	CtTTAttccaaatTcACT	204	138888	138907	>100%	0.03
ASO-002057	ASO-002057	ATTtCcaattcactTtTAC	98	138884	138903	>100%	0.03
ASO-002084	ASO-002084	ATTtCcaattcactTtTAC	95	138884	138903	>100%	0.03
ASO-002004	ASO-002004	CTTTatttccaaatTcACT	218	138889	138907	>100%	0.03
ASO-002025	ASO-002025	TAttTcCaaattcactTTTA	129	138885	138904	>100%	0.03
ASO-001942	ASO-001942	CtTtTAttccaaatTcACT	203	138888	138907	>100%	0.03
ASO-002035	ASO-002035	TAttTcCaaattcactTTTA	123	138885	138904	>100%	0.03
ASO-001967	ASO-001967	TTtTAttccaaatTcACT	188	138887	138906	>100%	0.04
ASO-002080	ASO-002080	TTtTAttccaaatTcACT	155	138886	138905	>100%	0.04

37/62

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
ASO-002076	ASO-002076	ATTCCaaattcacTTtAC	87	138884	138903	>100%	0.04
ASO-002090	ASO-002090	ATTtcCaaattcacTtTAC	85	138884	138903	>100%	0.04
ASO-002012	ASO-002012	CTTtatttccaaatTcACT	219	138889	138907	>100%	0.04
ASO-002083	ASO-002083	TAtTTcCaaattcacTtTTA	140	138885	138904	>100%	0.04
ASO-002037	ASO-002037	ATTTcCaaattcaCtTtTAC	96	138884	138903	>100%	0.04
ASO-002053	ASO-002053	TAtTtCcaaatcacTTTA	125	138885	138904	>100%	0.04
ASO-002002	ASO-002002	CTTtAttTccaaattcACTT	205	138888	138907	>100%	0.04
ASO-002071	ASO-002071	TTaTtTccaaattcacTTTT	154	138886	138905	89%	0.05
ASO-002033	ASO-002033	TATtTccaaattcacTtTTA	130	138885	138904	>100%	0.05
ASO-002008	ASO-002008	CTTtatttccaaatTCaCT	224	138889	138907	>100%	0.05
ASO-002094	ASO-002094	ATtTCcaaatcacTtTtAC	92	138884	138903	83%	0.05
ASO-002047	ASO-002047	TTATtccaaattCaCtTT	166	138886	138905	>100%	0.05
ASO-002066	ASO-002066	TTATtccaaattCacTtTT	164	138886	138905	96%	0.05
ASO-001933	ASO-001933	ATtTCcaaatcacTtTtAC	91	138884	138903	>100%	0.05
ASO-002039	ASO-002039	ATtTCcaaatcacTtTtAC	103	138884	138903	35%	0.05
ASO-001966	ASO-001966	TAtTTcCaaattcacTtTtA	128	138885	138904	>100%	0.05
ASO-002026	ASO-002026	TTatTTccaaattcaCtTTT	238	138886	138905	>100%	0.06
ASO-002054	ASO-002054	TTaTTtccaaattcaCtTTT	161	138886	138905	81%	0.06
ASO-002074	ASO-002074	TTatTTcCaaattcaCtTtTT	159	138886	138905	89%	0.06
ASO-002045	ASO-002045	TTaTtCcaaatcacTTTT	157	138886	138905	>100%	0.06
ASO-002011	ASO-002011	CTtTatttccaaatTcAC	227	138890	138907	>100%	0.06
ASO-002073	ASO-002073	TAtTtCcaaatcacTtTTA	135	138885	138904	>100%	0.06
ASO-001994	ASO-001994	CTtTatttccaaattcACTT	209	138888	138907	45%	0.06
ASO-002089	ASO-002089	TATTtccaaattcaCtTTA	134	138885	138904	>100%	0.06
ASO-002048	ASO-002048	TATtTccaaattcaCtTTA	124	138885	138904	>100%	0.07
ASO-001997	ASO-001997	CTTtatttccaaatTcAC	230	138890	138907	>100%	0.07
ASO-002091	ASO-002091	TTaTtTccaaattcaCtTTT	162	138886	138905	>100%	0.07
ASO-002056	ASO-002056	TATTtCcaaatcacTtTtA	132	138885	138904	>100%	0.07

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
ASO-002081	ASO-002081	TATtCcaattcacTtTA	127	138885	138904	>100%	0.07
ASO-001961	ASO-001961	TtTATTccaattcAcTtT	191	138887	138906	83%	0.07
ASO-002067	ASO-002067	TAtTccaattcaCTtTA	126	138885	138904	>100%	0.07
ASO-001960	ASO-001960	TaTTTccaattcacTtTA	131	138885	138904	>100%	0.08
ASO-002028	ASO-002028	TAtTccaattcaCttTA	138	138885	138904	77%	0.08
ASO-002063	ASO-002063	TATtCcaattcacTtTA	133	138885	138904	>100%	0.08
ASO-002064	ASO-002064	TtAtTccaattcaCtTtT	163	138886	138905	>100%	0.09
ASO-002085	ASO-002085	TAtTccaattcaCtTtTA	139	138885	138904	>100%	0.09
ASO-002055	ASO-002055	TAtTccaattcacTtTA	122	138885	138904	>100%	0.09
ASO-002027	ASO-002027	TATtTccaattcaCtTtTA	136	138885	138904	>100%	0.09
ASO-002093	ASO-002093	TTAtTccaattcAcTtT	160	138886	138905	>100%	0.09
ASO-002022	ASO-002022	TTAtTccaattcaCtTtT	237	138886	138905	>100%	0.09
ASO-002017	ASO-002017	CTtTATTccaattcaCTT	202	138888	138907	>100%	0.10
ASO-001941	ASO-001941	TtAtTccaattcaCtTtT	156	138886	138905	>100%	0.10
ASO-002044	ASO-002044	TTAtTccaattcaCtTtT	165	138886	138905	>100%	0.11
ASO-002046	ASO-002046	TTAtTccaattcacTtTtT	167	138886	138905	>100%	0.11
ASO-002005	ASO-002005	CTtTattccaattcaCTT	207	138888	138907	>100%	0.11
ASO-002024	ASO-002024	TTAtTccaattcaCtTtT	239	138886	138905	>100%	0.11
ASO-002016	ASO-002016	CTTtattccaattTcaCT	226	138889	138907	>100%	0.12
ASO-001998	ASO-001998	CTtTAttccaattcACTT	208	138888	138907	75%	0.13
ASO-002072	ASO-002072	TTAtTccaattcaCtTtT	153	138886	138905	>100%	0.13
ASO-001934	ASO-001934	TtATTccaattcaCtTtT	158	138886	138905	70%	0.13
ASO-002010	ASO-002010	CTTtattccaattTcaCTT	206	138888	138907	>100%	0.14
ASO-002075	ASO-002075	TATtTccaattcaCtTtTA	137	138885	138904	>100%	0.14
ASO-002013	ASO-002013	CTTtAttccaattcaCTT	210	138888	138907	30%	0.14
ASO-002020	ASO-002020	AcTTTattccaattcactTTtaC	187	138884	138908	18%	0.18
ASO-002049	ASO-002049	ACTTtattccaattcactTTtaC	240	138884	138908	29%	0.20

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
ASO-002086	ASO-002086	AcTTatttccaaattcactTTTaC	189	138884	138908	77%	0.25
ASO-002029	ASO-002029	AcTTatttccaaattcactTTTaC	190	138884	138908	29%	0.37
ASO-002019	ASO-002019	AcTTatttccaaattcactTTTaC	241	138884	138909	18%	0.39
ASO-002068	ASO-002068	AcTTatttccaaattcactTTTaC	194	138884	138908	35%	0.43
ASO-002009	ASO-002009	CTTAttccaaattcactCTT	211	138888	138907	>100%	0.49
ASO-002021	ASO-002021	AcTTatttccaaattcactTTTaC	193	138884	138908	24%	0.64
ASO-002095	ASO-002095	AcTTatttccaaattcactTTTaC	192	138884	138908	18%	0.67
ASO-000540-11	ASO-002155	CCCTtaatttcacCcTC	44	136053	136069		
ASO-000540-13	ASO-002179	CCCTtaatttcacCcTC	45	136053	136069		
ASO-000540-14	ASO-002191	CCCTtaATTtccacctCA	46	136052	136068		
ASO-000540-16	ASO-002120	CCCTtaaTttcacctCA	47	136052	136068		
ASO-000540-17	ASO-002132	CCCTtaAttccacctCA	48	136052	136068		
ASO-000540-18	ASO-002144	CCCTtaATTtccacctCA	49	136052	136068		
ASO-000540-19	ASO-002156	CCCTtaATTtccacctCA	50	136052	136068		
ASO-000540-20	ASO-002168	CCCTtaatttcacctCA	51	136052	136068		
ASO-000540-22	ASO-002192	CCCTtaatttcacctCA	2	136052	136068		
ASO-000540-23	ASO-002109	CCCTAatttcacCctCA	3	136052	136068		
ASO-000540-24	ASO-002121	TcCCTtaatttcacctCT	4	136054	136070		
ASO-000540-25	ASO-002133	TcCCTtaatttcacctCT	5	136054	136070		
ASO-000540-26	ASO-002145	TcCCTtaatttcAccCT	6	136054	136070		
ASO-000540-27	ASO-002157	TcCCTtaatttcacctCT	7	136054	136070		
ASO-000540-28	ASO-002169	TcCCTtaatttcacctCT	8	136054	136070		
ASO-000540-3	ASO-002154	CCCTtaatttcacCcTC	9	136053	136069		
ASO-000540-30	ASO-002193	TCCCTtaatttcacctCT	71	136054	136070		
ASO-000540-31	ASO-002110	TCCCTtaatttcAccCT	72	136054	136070		
ASO-000540-32	ASO-002122	TCCCTtaatttcacctCT	73	136054	136070		
ASO-000540-34	ASO-002146	TCCCTtaatttcacCcCT	74	136054	136070		
ASO-000540-36	ASO-002170	TCCCTtaatttcCaccCT	265	136054	136070		

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
ASO-000540-39	ASO-002111	CCCTtaatttcacCctCA	75	136052	136069		
ASO-000540-4	ASO-002166	CCCtTaatttcacCccTC	76	136053	136069		
ASO-000540-40	ASO-002123	CCCtTaatttcacCctCA	77	136052	136069		
ASO-000540-41	ASO-002135	CCCTtaatttcAcCctCA	78	136052	136069		
ASO-000540-42	ASO-002147	CCCTtaatttcacccTCA	267	136052	136069		
ASO-000540-43	ASO-002159	CCCTtaatttcacccCtCA	10	136052	136069		
ASO-000540-45	ASO-002183	CCCTtaatttcacCctCA	11	136052	136069		
ASO-000540-46	ASO-002195	CCCTtaatttcacCtCA	12	136052	136069		
ASO-000540-47	ASO-002196	CCCTtaatttcacCctCA	13	136052	136069		
ASO-000540-48	ASO-002200	CCCTtaatttcacCccTCA	14	136052	136069		
ASO-000540-49	ASO-002204	CCCTtaatttcacCctCA	15	136052	136069		
ASO-000540-5	ASO-002178	CCCtTaatttcAcccTC	16	136053	136069		
ASO-000540-50	ASO-002208	CCCTtaatttcAccCtCA	17	136052	136069		
ASO-000540-51	ASO-002212	CCCTtaatttcAcCctCA	18	136052	136069		
ASO-000540-52	ASO-002216	TcCCTtaatttcacCcTC	19	136053	136070		
ASO-000540-53	ASO-002220	TcCCTtaatttcacccTC	20	136053	136070		
ASO-000540-54	ASO-002224	TcCCTtaatttcacCcTC	21	136053	136070		
ASO-000540-56	ASO-002201	TCCcttaatttcacCcTC	52	136053	136070		
ASO-000540-57	ASO-002205	TCCcTtaatttcacccTC	53	136053	136070		
ASO-000540-58	ASO-002209	TCCcTtaatttcacCcTC	54	136053	136070		
ASO-000540-59	ASO-002213	TCCcTtaatttcacCcTC	55	136053	136070		
ASO-000540-6	ASO-002190	CCCtTaatttcAcCcTC	56	136053	136069		
ASO-000540-60	ASO-002217	TCCcTtaatttcAcCcTC	57	136053	136070		
ASO-000540-61	ASO-002221	TCCCTtaatttcacCCTC	58	136053	136070		
ASO-000540-62	ASO-002225	TCCCTtaatttcacCtTC	59	136053	136070		
ASO-000540-63	ASO-002198	TCCCTtaatttcacCcTC	60	136053	136070		
ASO-000540-64	ASO-002202	TCCCTtaatttcAccCtTC	61	136053	136070		
ASO-000540-65	ASO-002206	TCCCTtaatttcCaccCtTC	62	136053	136070		

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
ASO-000540-66	ASO-002210	TCcTTaatttcacCctCA	63	136052	136070		
ASO-000540-68	ASO-002218	TCCcTTaatttcaccCtCA	64	136052	136070		
ASO-000540-69	ASO-002222	TCCcTTaatttcacCctCA	23	136052	136070		
ASO-000540-71	ASO-002199	TCCcTTaatttcacCcTCA	25	136052	136070		
ASO-000540-72	ASO-002203	TCCcTTaatttcacCCtCA	26	136052	136070		
ASO-000540-73	ASO-002207	TCCcTTaatttcacCcTCA	27	136052	136070		
ASO-000540-74	ASO-002211	TCCcTTaatttcACcCtCA	28	136052	136070		
ASO-000540-76	ASO-002219	TCCcTTaatttcacCCtCA	29	136052	136070		
ASO-000540-8	ASO-002119	CCcTTaatttcaccCTC	30	136053	136069		
ASO-000540-9	ASO-002131	CCcTTaatttcacCcTC	31	136053	136069		
ASO-001933-mm1	ASO-002291	GtTTCaaaattcactTTtAC	104	138884	138903		
ASO-001933-mm2	ASO-002303	AtTTCcagattcactTTtAC	105	138884	138903		
ASO-001933-mm3	ASO-002315	TtTTCcaaattcactTTtAC	106	138884	138903		
ASO-001933-mm4	ASO-002327	GtTTCcagattcactTTtAC	107	138884	138903		
ASO-001933-mm5	ASO-002339	AtTTCcaaagtctcactTTtGC	108	138884	138903		
ASO-001933-mm6	ASO-002351	AtTTCcagattcgctTTtAC	109	138884	138903		
ASO-001941-mm1	ASO-002317	AtTtccaaaattcaCTtTT	168	138886	138905		
ASO-001941-mm2	ASO-002329	TtAtTtccaaaattcaCTtTA	169	138886	138905		
ASO-001941-mm3	ASO-002341	TtAtTtccaaaattcaCTtTG	170	138886	138905		
ASO-001941-mm4	ASO-002353	AtTtTtccagattcaCTtTT	171	138886	138905		
ASO-001941-mm5	ASO-002365	TtAtTtccaaagtctcaCTtTC	172	138886	138905		
ASO-001941-mm6	ASO-002377	TtAtTtccagattcgCTtTT	173	138886	138905		

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
ASO-001942-mm1	ASO-002363	CTTATttcca <u>g</u> attCAcTT	212	138888	138907		
ASO-001942-mm2	ASO-002375	CTTATttccaaattCAcT <u>G</u>	213	138888	138907		
ASO-001942-mm3	ASO-002292	CTTATttccaaaattC <u>G</u> cTT	214	138888	138907		
ASO-001942-mm4	ASO-002304	CTTATttccagattCAcT <u>A</u>	215	138888	138907		
ASO-001942-mm5	ASO-002316	CTTATttccag <u>g</u> ttCAcTT	216	138888	138907		
ASO-001942-mm6	ASO-002328	CTTATttcc <u>g</u> attCAcTT	217	138888	138907		
ASO-001967-mm1	ASO-002294	<u>A</u> TTATttccaaaattCAcTT	195	138887	138906		
ASO-001967-mm2	ASO-002306	TTTATttccaa <u>g</u> ttCAcTT	196	138887	138906		
ASO-001967-mm3	ASO-002318	<u>G</u> TTATttccaaaattCAcTT	197	138887	138906		
ASO-001967-mm4	ASO-002330	<u>A</u> TTATttccagattCAcTT	198	138887	138906		
ASO-001967-mm5	ASO-002342	TTTATttcca <u>g</u> ttCAcTT	199	138887	138906		
ASO-001967-mm6	ASO-002354	<u>C</u> TTATttccaa <u>g</u> ttCAcTT	200	138887	138906		
ASO-001997-mm1	ASO-002320	CTTtatttcca <u>g</u> atTcAC	231	138890	138907		
ASO-001997-mm2	ASO-002332	CTTtatttcc <u>g</u> aatTcAC	232	138890	138907		
ASO-001997-mm3	ASO-002344	CTTt <u>g</u> tttccaaatTcAC	233	138890	138907		
ASO-001997-mm4	ASO-002356	CTTt <u>g</u> tttcca <u>g</u> atTcAC	234	138890	138907		
ASO-001997-mm5	ASO-002368	CTTtatttcca <u>g</u> gTcAC	235	138890	138907		
ASO-001997-mm6	ASO-002380	CTTt <u>g</u> tttccaa <u>g</u> TcAC	236	138890	138907		
SPC-15857-01	ASO-002326	ATTcCaattcacttTTAC	110	138884	138903		

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
SPC-15858-01	ASO-002338	ATTtcCaaattcactTTAC	111	138884	138903		
SPC-15859-01	ASO-002350	TATTTccaaattcactTTTA	141	138885	138904		
SPC-15860-01	ASO-002362	ATTtCcaaatcactTTAC	112	138884	138903		
SPC-15861-01	ASO-002374	TATTTccaaattcactTTTA	142	138885	138904		
SPC-15862-01	ASO-002386	TATTtccaaattcaCTtTA	143	138885	138904		
SPC-15863-01	ASO-002227	TATtTccaaattcactTTTA	144	138885	138904		
SPC-15864-01	ASO-002236	ATTTccaaattcacTtTTAC	113	138884	138903		
SPC-15865-01	ASO-002245	TAttTCcaaatcactTTTA	145	138885	138904		
SPC-15866-01	ASO-002253	TTAttTccaaattcaCTtTT	174	138886	138905		
SPC-15867-01	ASO-002261	TATtTccaaattcacTtTTA	146	138885	138904		
SPC-15868-01	ASO-002269	ATtTCcaaatcactTtTAC	114	138884	138903		
SPC-15869-01	ASO-002276	TATtTCcaaatcactTTTA	147	138885	138904		
SPC-15870-01	ASO-002283	TTAttTccaaattcacTTTT	175	138886	138905		
SPC-15871-01	ASO-002228	TATTtccaaattcaCTtTA	148	138885	138904		
SPC-15872-01	ASO-002237	ATtTCcaaatcactTTAC	115	138884	138903		
SPC-15873-01	ASO-002246	ATTtCcaaatcactTTtAC	116	138884	138903		
SPC-15874-01	ASO-002254	ATTTccaaattcacTtTAC	117	138884	138903		
SPC-15876-01	ASO-002270	TTATtccaaattcaCTTTT	176	138886	138905		
SPC-15877-01	ASO-002277	TTATtccaaattcACtTT	177	138886	138905		
SPC-15878-01	ASO-002284	ATtTccAaatcactTTAC	118	138884	138903		
SPC-15880-01	ASO-002238	ATTTccaaattcactTtTAC	119	138884	138903		
SPC-15881-01	ASO-002247	TTaTTtccaaattcacTTTT	178	138886	138905		
SPC-15883-01	ASO-002263	ATtTcCaaattcactTtTAC	120	138884	138903		
SPC-15884-01	ASO-002271	TTATtccaaattcacTTTT	179	138886	138905		
SPC-15885-01	ASO-002278	TTaTTtccaaattcACtTT	180	138886	138905		
SPC-15886-01	ASO-002285	TATTtccaaattcacTtTtTA	149	138885	138904		
SPC-15887-01	ASO-002230	TATTtccaaattcacTtTTA	150	138885	138904		
SPC-15888-01	ASO-002239	ATTTccaaattcactTtTAC	121	138884	138903		

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
SPC-15889-01	ASO-002248	TTaTTtccaaattcActTTT	181	138886	138905		
SPC-15890-01	ASO-002256	TATTtccaaattcActTtTA	151	138885	138904		
SPC-15891-01	ASO-002264	TTAtTtccaaattcActTTT	182	138886	138905		
SPC-15892-01	ASO-002272	TTaTTtccaaattcActTtT	183	138886	138905		
SPC-15893-01	ASO-002279	TATTtccaaattcActTtTA	152	138885	138904		
SPC-15894-01	ASO-002286	TTAtTtccaaattcActTtT	184	138886	138905		
SPC-15895-01	ASO-002231	TTATtccaaattcActTTT	185	138886	138905		
SPC-15896-01	ASO-002240	TTATtccaaattcActTtT	186	138886	138905		
TBD-mm10	ASO-002382	CCttgATttcgcctCA	32	136053	136069		
TBD-mm11	ASO-002299	CCttgATttcaccctCT	33	136052	136068		
TBD-mm12	ASO-002311	CCttaGTTtcaccctCG	34	136054	136070		
TBD-mm13	ASO-002323	TcCCtTgatttcaccCT	79	136053	136069		
TBD-mm14	ASO-002335	TcCCtTaatttcaccCA	80	136052	136068		
TBD-mm15	ASO-002347	TcCCtTaatttcgaccCT	81	136053	136069		
TBD-mm16	ASO-002359	TcCCtTgatttcaccCA	82	136053	136069		
TBD-mm17	ASO-002371	TcCCtTgatttcaccCG	83	136052	136068		
TBD-mm18	ASO-002383	TcCCtTgatttcgaccCT	84	136052	136068		
TBD-mm25	ASO-002372	TcCCtTgatttcacCcTC	65	136052	136069		
TBD-mm26	ASO-002384	TcCCtTaatttcgCcTC	66	136052	136069		
TBD-mm27	ASO-002301	TcCCtTgatttcacCcTC	67	136052	136069		
TBD-mm28	ASO-002313	TcCCtTgatttcgCcTC	68	136054	136071		

Figure 3 (cont.)

Oligomer Name	ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	Oscillation data % control	IC ₅₀ (μM) hTau neurons
TBD-mm29	ASO-002325	TcCCTTggtttcgcCctC	69	136052	136069		
TBD-mm30	ASO-002337	AcCCCTtgatttcacCcTC	70	136053	136070		
TBD-mm31	ASO-002349	TcCCTtgatttcacCctCA	35	136053	136071		
TBD-mm32	ASO-002361	TcCCTtaatttcacCctCG	36	136052	136070		
TBD-mm33	ASO-002373	AcCCTtaatttcacCctCA	37	136053	136071		
TBD-mm34	ASO-002385	TcCCTtgatttcgcCctCA	38	136052	136070		
TBD-mm35	ASO-002302	TcCCTTggtttcacCctCG	39	136054	136072		
TBD-mm36	ASO-002314	AcCCTtgatttcacCctCA	40	136054	136072		
TBD-mm7	ASO-002346	CCttgATttcacctCA	41	136053	136069		
TBD-mm8	ASO-002358	CCttaGTTtcacctCA	42	136053	136069		
TBD-mm9	ASO-002370	CCttaATttcacctCG	43	136052	136068		
"mm"	indicates mismatches.	The	mismatched	bases	are		underlined.

Figure 4

ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	<i>In vivo</i> acute tolerability score	brain tau mRNA % remaining
ASO-001941	TTaTTtccaaattcaCTtTT	156	138886	138905	2.00	14
ASO-001942	CTtTAtttccaaattCacTT	203	138888	138907	2.00	16
ASO-001935	CtTTAtttccaaattCacTT	204	138888	138907	1.33	19
ASO-001933	AtTTCcaaattcactTTtAC	91	138884	138903	0.83	20
ASO-001967	TTtATttccaaattcAcTT	188	138887	138906	1.00	22
ASO-001960	TaTTTccaaattcacTTtTA	131	138885	138904	5.00	23
ASO-001966	TAtTTccaaattcacTTtTA	128	138885	138904	4.33	23
ASO-001961	TtTATttccaaattcAcTT	191	138887	138906	2.17	25
ASO-001934	TtATTtccaaattcaCTtTT	158	138886	138905	0.67	43
ASO-001940	ATtTCcaaattcactTTtAC	90	138884	138903	0.29	
ASO-001997	CTTtatttccaaatTcAC	230	138890	138907		
ASO-001998	CTttAtttccaaattcACTT	208	138888	138907		
ASO-002002	CTTtAtttccaaattcACTT	205	138888	138907		
ASO-002005	CTtTatttccaaattcaCTT	207	138888	138907		
ASO-002008	CTTtatttccaaatTcaCT	224	138889	138907		
ASO-002009	CTttAtttccaaattcaCTT	211	138888	138907		
ASO-002010	CTTtatttccaaatTcaCTT	206	138888	138907		
ASO-002011	CTtTatttccaaatTcAC	227	138890	138907		
ASO-002012	CTTtatttccaaatTcaCT	219	138889	138907		
ASO-002016	CTTtatttccaaatTcaCT	226	138889	138907		
ASO-002022	TTATttccaaattcaCTtTT	237	138886	138905		
ASO-002023	ATTtCcaaattcacTTtAC	100	138884	138903		
ASO-002024	TTAttTccaaattcaCtTTT	239	138886	138905		
ASO-002025	TAttTcCaaattcactTTTA	129	138885	138904		
ASO-002026	TTatTTccaaattcaCtTTT	238	138886	138905		
ASO-002027	TATtTccaaattcaCtTtTA	136	138885	138904		
ASO-002028	TAtTTccaaattcaCttTTA	138	138885	138904		
ASO-002033	TATtTccaaattcacTtTTA	130	138885	138904		
ASO-002034	ATTtCcaaattcactTtTAC	93	138884	138903		
ASO-002035	TAtTtcCaaattcactTTTA	123	138885	138904		
ASO-002036	ATtTcCaAattcacttTTAC	94	138884	138903		
ASO-002037	ATTtCcaaattcaCtTtAC	96	138884	138903		
ASO-002038	ATTtCcaaattcacTtTAC	102	138884	138903		
ASO-002043	ATtTCcaaattcacttTTAC	86	138884	138903		
ASO-002044	TTAtTtccaaattcaCtTTT	165	138886	138905		
ASO-002045	TTaTttCcaaattcacTTTT	157	138886	138905		
ASO-002046	TTatTtCcaaattcacTTTT	167	138886	138905		
ASO-002047	TTATttccaaattCaCttTT	166	138886	138905		
ASO-002048	TATtTccaaattcaCttTTA	124	138885	138904		
ASO-002053	TAtTtCcaaattcactTTTA	125	138885	138904		

Figure 4 (cont.)

ASO Number	ASO Sequence	SEQ ID NO:	premRNA start NG_007398	premRNA end NG_007398	<i>In vivo</i> acute tolerability score	brain tau mRNA % remaining
ASO-002054	TTaTTtcaaattcaCtTTT	161	138886	138905		
ASO-002055	TAtTtCcaaattcacTTtTA	122	138885	138904		
ASO-002056	TAttTCcaaattcacTTtTA	132	138885	138904		
ASO-002057	ATTTcaaattcaCttTtAC	98	138884	138903		
ASO-002058	ATTtCcaaattcacTttTAC	97	138884	138903		
ASO-002062	ATTtcCaaattcactTTtAC	88	138884	138903		
ASO-002063	TATtTcaaattcacTTtTA	133	138885	138904		
ASO-002064	TTaTtTcaaattcaCTtTT	163	138886	138905		
ASO-002065	ATtTCcaaattcactTTtAC	101	138884	138903		
ASO-002066	TTATttcaaattCacTtTT	164	138886	138905		
ASO-002067	TATTTcaaattcaCTtTA	126	138885	138904		
ASO-002071	TTaTtTcaaattcacTTTT	154	138886	138905		
ASO-002072	TTAttTcaaattcaCTtTT	153	138886	138905		
ASO-002073	TAtTtCcaaattcacTtTTA	135	138885	138904		
ASO-002074	TTatTTcaaattcaCTtTT	159	138886	138905		
ASO-002075	TATtTcaaattcaCTtTA	137	138885	138904		
ASO-002076	ATtTCcaaattcacTTtAC	87	138884	138903		
ASO-002080	TTatTTcaaattcacTTTT	155	138886	138905		
ASO-002081	TATtTcaaattcacTtTTA	127	138885	138904		
ASO-002082	ATtTCaaattcactTtTAC	89	138884	138903		
ASO-002083	TAttTCcaaattcacTtTTA	140	138885	138904		
ASO-002084	ATTtCcaaattcacTTtAC	95	138884	138903		
ASO-002085	TATTTcaaattcaCtTtTA	139	138885	138904		
ASO-002086	AcTTtatttcaaattcactTTT aC	189	138884	138908		
ASO-002089	TATTtcaaattcaCttTTA	134	138885	138904		
ASO-002090	ATTtcCaaattcactTtTAC	85	138884	138903		
ASO-002091	TTaTtTcaaattcaCtTTT	162	138886	138905		
ASO-002092	ATtTCaaattcactTtTAC	99	138884	138903		
ASO-002093	TTAtTtcaaattcACtTT	160	138886	138905		
ASO-002094	ATtTCaaattcacTtTtAC	92	138884	138903		

Figure 5

RhoA (SEQ ID NO:267)	a	c	t	t	t	a	t	a	t	a	a	a	a	c	c	c	t	t	a	t	a	c	c	t	t	c	t	t		
ASO Sequence																														
ASO Number (SEQ ID NO:.)																											CRC hTau hTau (nM)	Tuj 1 Screening 5uM (% inhibition)	Rho CDI neurons 5uM (% inhibition)	4 wk+ tolerability
ASO-000757 (268)																											12	77%	74%	No
ASO-0001967 (269)																											36	13%	20%	Yes
ASO-000755 (270)																											34	53%	25%	No
ASO-001941 (156)																											102	30%	22%	Yes
ASO-000753 (271)																											110	72%	30%	No
ASO-002038 (102)																											24	0%	11%	Yes
ASO-001933 (91)																											53	27%	12%	Yes
ASO-001940 (90)																											27	10%	0%	Yes

Figure 6A

ASO No.	SEQ ID NO:	Sequence	tau Human Transcript start (SEQID NO.: 1)	Human Transcript tau end (SEQID NO.: 1)	hTau Neurons Tau HC IC50 (uM)	Tau % inhibition (htau neurons)
ASO-001936	272	CcAGCgtgatcttccATcAC	72,944	72,963		
ASO-001937	273	TcCCAgcgtgatcttCCaTC	72,946	72,965		
ASO-001943	274	CCaGcgtgatcttccATcAC	72,944	72,963		
ASO-001944	275	TCcCAgcgtgatcttCCaTC	72,946	72,965		
ASO-001963	276	CcCAGcgtgatcttcCAtCA	72,945	72,964	1.05	
ASO-001964	277	CgTCCcagcgtgatcTTcCA	72,948	72,967		
ASO-001969	278	CCcAGcgtgatcttcCAtCA	72,945	72,964	0.888	
ASO-001970	279	CGtCCcagcgtgatcTTcCA	72,948	72,967		
ASO-002100	280	CATCacttcgaaCtCC	72,934	72,949	0.01782	99.92
ASO-002101	281	CCatcacttCGaacTC	72,935	72,950	0.09601	99.62
ASO-002102	282	CaTCACttcgaacTcCT	72,933	72,949	0.07167	98.90
ASO-002103	283	TCCatCacttcgaaCTC	72,935	72,951	0.1482	98.01
ASO-002104	284	CttCcatcacttCGaaCT	72,936	72,953	0.2187	99.48
ASO-002105	285	CtTcCATcacttcgaaCTCC	72,934	72,953	0.973	75.93
ASO-002106	286	TCTtcCATcacttcgaacTCCT	72,933	72,934	0.4949	94.21
ASO-002112	287	CATcActtcgaaCtCC	72,934	72,949	0.02334	99.68
ASO-002113	288	CCatcacttCGaaCTC	72,935	72,950	0.083	99.87
ASO-002114	289	CCaTCacttcgaaCtCC	72,934	72,950	0.1991	97.06
ASO-002115	290	TcCATcacttcgaaCTC	72,935	72,951	0.04368	99.59
ASO-002116	291	TCCatCacttcgaaCTCC	72,934	72,951	1.308	72.30
ASO-002125	292	CcAtCActtcgaaCTC	72,935	72,950	0.09573	98.01
ASO-002126	293	CCaTCacttcgaacTCC	72,934	72,950	0.08785	99.50
ASO-002128	294	TCcAtCacttcgaaCtCC	72,934	72,951	0.2319	96.38
ASO-002129	295	TcTtCcatcacttCGaaCTC	72,935	72,954	0.1438	99.17
ASO-002136	296	CATCacTtcgaactCC	72,934	72,949	0.00935	98.94
ASO-002137	297	CcaTCacttcgaaCTC	72,935	72,950	0.02104	99.91
ASO-002138	298	CCaTcACttcgaacTCC	72,934	72,950	0.05625	98.72
ASO-002139	299	CCATcacttcgaaCTcCT	72,933	72,950	0.2047	98.88
ASO-002141	300	TcTtCcatcacttCGaaCTC	72,935	72,954	0.09552	99.31
ASO-002149	301	CcatCActtcgaaCTC	72,935	72,950	0.04234	99.81
ASO-002150	302	CcaTCaCttcgaactCC	72,934	72,950	0.01932	99.92
ASO-002151	303	CcATcACttcgaacTcCT	72,933	72,950	0.02866	99.72
ASO-002153	304	TTCCatCacttcgaaCtCCT	72,933	72,952	5.374	39.88
ASO-002160	305	CATcAcTtcgaactCC	72,934	72,949	0.01106	99.91
ASO-002161	306	CATCaCttcgaacTcCT	72,933	72,949	0.09007	99.31
ASO-002164	307	TtCCatcacttCGaaCTC	72,935	72,952	0.1231	95.37
ASO-002165	308	TtCCAtCacttcgaaCtCCT	72,933	72,952	2.614	58.46
ASO-002172	309	CATCaCTtcgaactCC	72,934	72,949	0.01604	99.83

Figure 6A (cont.)

ASO-002173	310	CATcACttcgaacTcCT	72,933	72,949	0.01512	99.90
ASO-002174	311	TCCatcacttCGaaCTC	72,935	72,951	0.2206	98.99
ASO-002176	312	TtCCatcacttCgaaCTC	72,935	72,952	0.06522	96.99
ASO-002177	313	TtCCaTCacttcgaaCtCCT	72,933	72,952	2.995	52.25
ASO-002184	314	CCatcacttCGaaCTC	72,935	72,950	0.2095	99.41
ASO-002185	315	CAtCACttcgaacTcCT	72,933	72,949	0.03681	99.90
ASO-002186	316	TCCatcacttCgaaCTC	72,935	72,951	0.04436	98.20
ASO-002187	317	CttCcatcacttCGaaCT	72,936	72,953	0.1427	99.87

Figure 6B

ASO No. (SEQ ID NO:)	Sequence	tau Human Transcrip t start (SEQID NO.: 1)	Human Transcript tau end (SEQID NO.: 1)	Structure
ASO- 001936 (272)	CcAGCgtgatcttc ATcAC	72,944	72,963	OxyMCs DNACs OxyAs OxyGs OxyMCs DNAGs DNAts DNAGs DNAas DNAts DNACs DNAts DNAts DNACs DNACs OxyAs OxyTs DNACs OxyAs OxyMC
ASO- 001937 (273)	TcCCAgcgtgatctt CCaTC	72,946	72,965	OxyTs DNACs OxyMCs OxyMCs OxyAs DNAGs DNACs DNAGs DNAts DNAGs DNAas DNAts DNACs DNAts DNAts OxyMCs OxyMCs DNAas OxyTs OxyMC
ASO- 001943 (274)	CCaGCgtgatcttc ATcAC	72,944	72,963	OxyMCs OxyMCs DNAas OxyGs OxyMCs DNAGs DNAts DNAGs DNAas DNAts DNACs DNAts DNAts DNACs DNACs OxyAs OxyTs DNACs OxyAs OxyMC
ASO- 001944 (275)	TCcCAGcgtgatctt CCaTC	72,946	72,965	OxyTs OxyMCs DNACs OxyMCs OxyAs DNAGs DNACs DNAGs DNAts DNAGs DNAas DNAts DNACs DNAts DNAts OxyMCs OxyMCs DNAas OxyTs OxyMC
ASO- 001963 (276)	CcCAGcgtgatcttc CAcCA	72,945	72,964	OxyMCs DNACs OxyMCs OxyAs OxyGs DNACs DNAGs DNAts DNAGs DNAas DNAts DNACs DNAts DNAts DNACs OxyMCs OxyAs DNAts OxyMCs OxyA
ASO- 001964 (277)	CgTCCcagcgtgatc TTcCA	72,948	72,967	OxyMCs DNAGs OxyTs OxyMCs OxyMCs DNACs DNAas DNAGs DNACs DNAGs DNAts DNAGs DNAas DNAts DNACs OxyTs OxyTs DNACs OxyMCs OxyA
ASO- 001969 (278)	CCcAGcgtgatcttc CAcCA	72,945	72,964	OxyMCs OxyMCs DNACs OxyAs OxyGs DNACs DNAGs DNAts DNAGs DNAas DNAts DNACs DNAts DNAts DNACs OxyMCs OxyAs DNAts OxyMCs OxyA
ASO- 001970 (279)	CGtCCcagcgtgatc TTcCA	72,948	72,967	OxyMCs OxyGs DNAts OxyMCs OxyMCs DNACs DNAas DNAGs DNACs DNAGs DNAts DNAGs DNAas DNAts DNACs OxyTs OxyTs DNACs OxyMCs OxyA
ASO- 001978 (318)	TCCcagcgtgatcttc cATC	72,946	72,965	OxyTs OxyMCs OxyMCs DNACs DNAas DNAGs DNACs DNAGs DNAts DNAGs DNAas DNAts DNACs DNAts DNAts DNACs DNACs OxyAs OxyTs OxyMC
ASO- 002100 (280)	CATCacttcgaaCtC C	72,934	72,949	OxyMCs OxyAs OxyTs OxyMCs DNAas DNACs DNAts DNAts DNACs DNAGs DNAas DNAas OxyMCs DNAts OxyMCs OxyMC
ASO- 002101 (281)	CCatcacttCGaacT C	72,935	72,950	OxyMCs OxyMCs DNAas DNAts DNACs DNAas DNACs DNAts DNAts OxyMCs OxyGs DNAas DNAas DNACs OxyTs OxyMC
ASO- 002102 (282)	CaTCACttcgaacT cCT	72,933	72,949	OxyMCs DNAas OxyTs OxyMCs OxyAs OxyMCs DNAts DNAts DNACs DNAGs DNAas DNAas DNACs OxyTs DNACs OxyMCs OxyT

Figure 6B (cont.)

ASO-002103 (283)	TCCatCacttcgaaC TC	72,935	72,951	OxyTs OxyMCs OxyMCs DNAas DNAts OxyMCs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002104 (284)	CttCcatcacttCgaa CT	72,936	72,953	OxyMCs DNAts DNAts OxyMCs DNacs DNAas DNAts DNacs DNAas DNacs DNAts DNAts OxyMCs DNags DNAas DNAas OxyMCs OxyT
ASO-002105 (285)	CtTcCATcacttcga aCTCC	72,934	72,953	OxyMCs DNAts OxyTs DNacs OxyMCs OxyAs OxyTs DNacs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs OxyTs OxyMCs OxyMC
ASO-002106 (286)	TCTtcCATcacttcg aacTCCT	72,933	72,934	OxyTs OxyMCs OxyTs DNAts DNacs OxyMCs OxyAs DNAts DNacs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas DNacs OxyTs OxyMCs OxyMCs OxyT
ASO-002112 (287)	CAtCActtcgaaCtC C	72,934	72,949	OxyMCs OxyAs DNAts OxyMCs OxyAs DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs DNAts OxyMCs OxyMC
ASO-002113 (288)	CCatcacttCgaaCT C	72,935	72,950	OxyMCs OxyMCs DNAas DNAts DNacs DNAas DNacs DNAts DNAts OxyMCs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002114 (289)	CCaTCacttcgaaCt CC	72,934	72,950	OxyMCs OxyMCs DNAas OxyTs OxyMCs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs DNAts OxyMCs OxyMC
ASO-002115 (290)	TcCATcacttcgaaC TC	72,935	72,951	OxyTs DNacs OxyMCs OxyAs OxyTs DNacs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002116 (291)	TCCatCacttcgaaC TCC	72,934	72,951	OxyTs OxyMCs OxyMCs DNAas DNAts OxyMCs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs OxyTs OxyMCs OxyMC
ASO-002125 (292)	CcAtCActtcgaaCT C	72,935	72,950	OxyMCs DNacs OxyAs DNAts OxyMCs OxyAs DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002126 (293)	CCaTCacttcgaaCT CC	72,934	72,950	OxyMCs OxyMCs DNAas OxyTs OxyMCs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas DNacs OxyTs OxyMCs OxyMC
ASO-002128 (294)	TCcAtCacttcgaaC tCC	72,934	72,951	OxyTs OxyMCs DNacs OxyAs DNAts OxyMCs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs DNAts OxyMCs OxyMC
ASO-002129 (295)	TCttCcatcacttCG aaCTC	72,935	72,954	OxyTs OxyMCs DNAts DNAts OxyMCs DNacs DNAas DNAts DNacs DNAas DNacs DNAts DNAts OxyMCs OxyGs DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002136 (296)	CATCacTtcgaact CC	72,934	72,949	OxyMCs OxyAs OxyTs OxyMCs DNAas DNacs OxyTs DNAts DNacs DNags DNAas DNAas DNacs DNAts OxyMCs OxyMC
ASO-002137 (297)	CcaTCacttcgaaCT C	72,935	72,950	OxyMCs DNacs DNAas OxyTs OxyMCs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs OxyTs OxyMC

Figure 6B (cont.)

ASO-002138 (298)	CCaTcACttcgaac TCC	72,934	72,950	OxyMCs OxyMCs DNAas OxyTs DNacs OxyAs OxyMCs DNats DNats DNacs DNags DNAas DNAas DNacs OxyTs OxyMCs OxyMC
ASO-002139 (299)	CCATcacttcgaaC TcCT	72,933	72,950	OxyMCs OxyMCs OxyAs OxyTs DNacs DNAas DNacs DNats DNats DNacs DNags DNAas DNAas OxyMCs OxyTs DNacs OxyMCs OxyT
ASO-002141 (300)	TCttCcatcacttCga aCTC	72,935	72,954	OxyTs OxyMCs DNats DNats OxyMCs DNacs DNAas DNats DNacs DNAas DNacs DNats DNats OxyMCs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002149 (301)	CcatCActtcgaaCT C	72,935	72,950	OxyMCs DNacs DNAas DNats OxyMCs OxyAs DNacs DNats DNats DNacs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002150 (302)	CcaTCaCttcgaact CC	72,934	72,950	OxyMCs DNacs DNAas OxyTs OxyMCs DNAas OxyMCs DNats DNats DNacs DNags DNAas DNAas DNacs DNats OxyMCs OxyMC
ASO-002151 (303)	CcATcACttcgaac TcCT	72,933	72,950	OxyMCs DNacs OxyAs OxyTs DNacs OxyAs OxyMCs DNats DNats DNacs DNags DNAas DNAas DNacs OxyTs DNacs OxyMCs OxyT
ASO-002153 (304)	TTCCatCacttcgaa CtCCT	72,933	72,952	OxyTs OxyTs OxyMCs OxyMCs DNAas DNats OxyMCs DNAas DNacs DNats DNats DNacs DNags DNAas DNAas OxyMCs DNats OxyMCs OxyMCs OxyT
ASO-002160 (305)	CATcACttcgaactC C	72,934	72,949	OxyMCs OxyAs DNats OxyMCs OxyAs DNacs OxyTs DNats DNacs DNags DNAas DNAas DNacs DNats OxyMCs OxyMC
ASO-002161 (306)	CATCaCttcgaacT cCT	72,933	72,949	OxyMCs OxyAs OxyTs OxyMCs DNAas OxyMCs DNats DNats DNacs DNags DNAas DNAas DNacs OxyTs DNacs OxyMCs OxyT
ASO-002164 (307)	TtCCatcacttCGaa CTC	72,935	72,952	OxyTs DNats OxyMCs OxyMCs DNAas DNats DNacs DNAas DNacs DNats DNats OxyMCs OxyGs DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002165 (308)	TtCCATcacttcgaa CtCCT	72,933	72,952	OxyTs DNats OxyMCs OxyMCs OxyAs DNats OxyMCs DNAas DNacs DNats DNats DNacs DNags DNAas DNAas OxyMCs DNats OxyMCs OxyMCs OxyT
ASO-002172 (309)	CATCaCTtcgaactC C	72,934	72,949	OxyMCs OxyAs DNats OxyMCs DNAas OxyMCs OxyTs DNats DNacs DNags DNAas DNAas DNacs DNats OxyMCs OxyMC
ASO-002173 (310)	CATcACttcgaacT cCT	72,933	72,949	OxyMCs OxyAs OxyTs DNacs OxyAs OxyMCs DNats DNats DNacs DNags DNAas DNAas DNacs OxyTs DNacs OxyMCs OxyT
ASO-002174 (311)	TCCatcacttCGaa CTC	72,935	72,951	OxyTs OxyMCs OxyMCs DNAas DNats DNacs DNAas DNacs DNats DNats OxyMCs OxyGs DNAas DNAas OxyMCs OxyTs OxyMC

Figure 6B (cont.)

ASO-002176 (312)	TtCCatcacttCgaaCTC	72,935	72,952	OxyTs DNAts OxyMCs OxyMCs DNAas DNAts DNacs DNAas DNacs DNAts DNAts OxyMCs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002177 (313)	TtCCaTCacttcgaaCtCCT	72,933	72,952	OxyTs DNAts OxyMCs OxyMCs DNAas OxyTs OxyMCs DNAas DNacs DNAts DNAts DNacs DNags DNAas DNAas OxyMCs DNAts OxyMCs OxyMCs OxyT
ASO-002184 (314)	CCatcacttCGaaCTC	72,935	72,950	OxyMCs OxyMCs DNAas DNAts DNacs DNAas DNacs DNAts DNAts OxyMCs OxyGs DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002185 (315)	CAtCACttcgaacTcCT	72,933	72,949	OxyMCs OxyAs DNAts OxyMCs OxyAs OxyMCs DNAts DNAts DNacs DNags DNAas DNAas DNacs OxyTs DNacs OxyMCs OxyT
ASO-002186 (316)	TCCatcacttCgaaCTC	72,935	72,951	OxyTs OxyMCs OxyMCs DNAas DNAts DNacs DNAas DNacs DNAts DNAts OxyMCs DNags DNAas DNAas OxyMCs OxyTs OxyMC
ASO-002187 (317)	CttCcatcacttCGaaCT	72,936	72,953	OxyMCs DNAts DNAts OxyMCs DNacs DNAas DNAts DNacs DNAas DNacs DNAts DNAts OxyMCs OxyGs DNAas DNAas OxyMCs OxyT

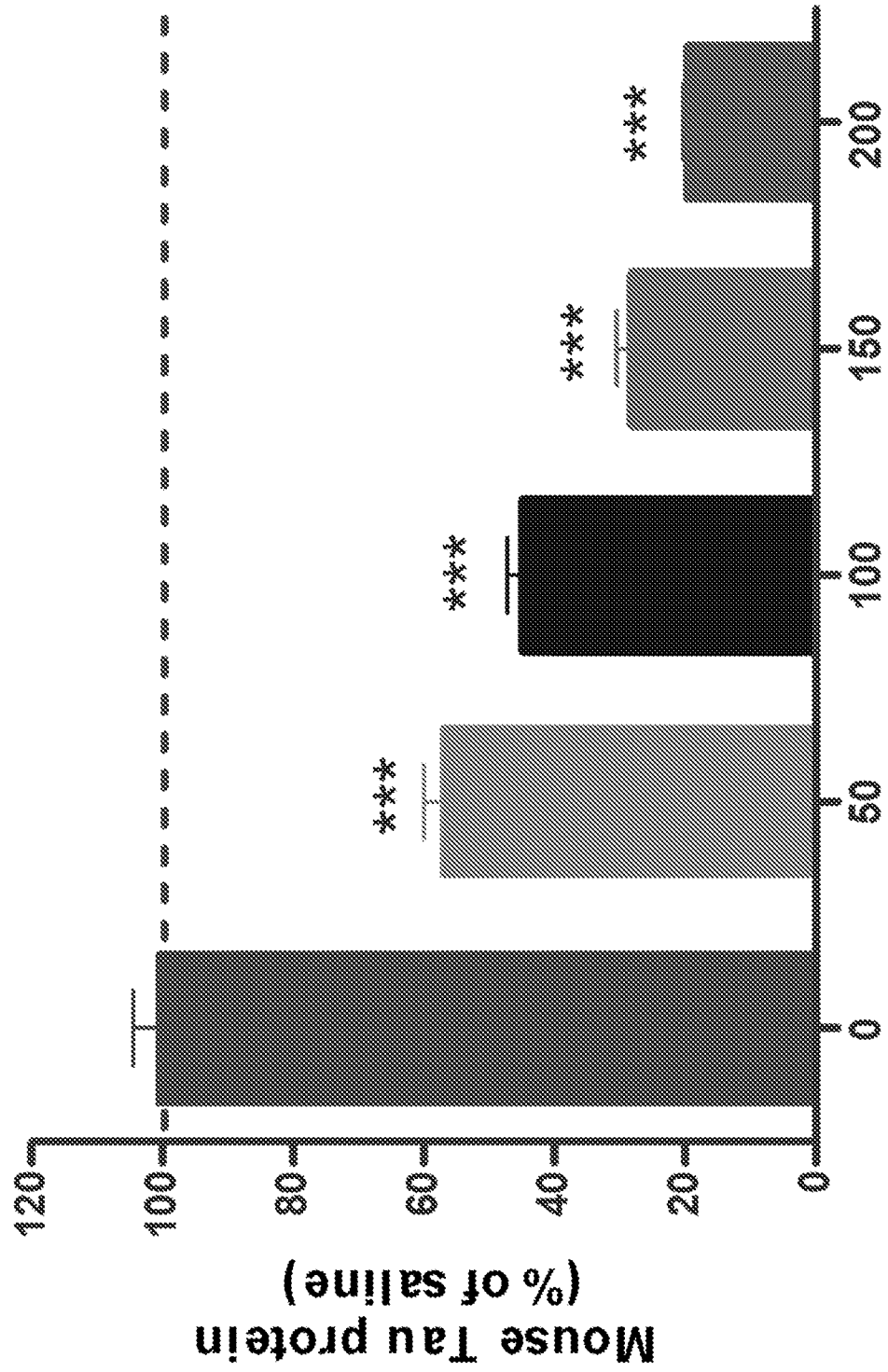


Figure 7

Figure 8

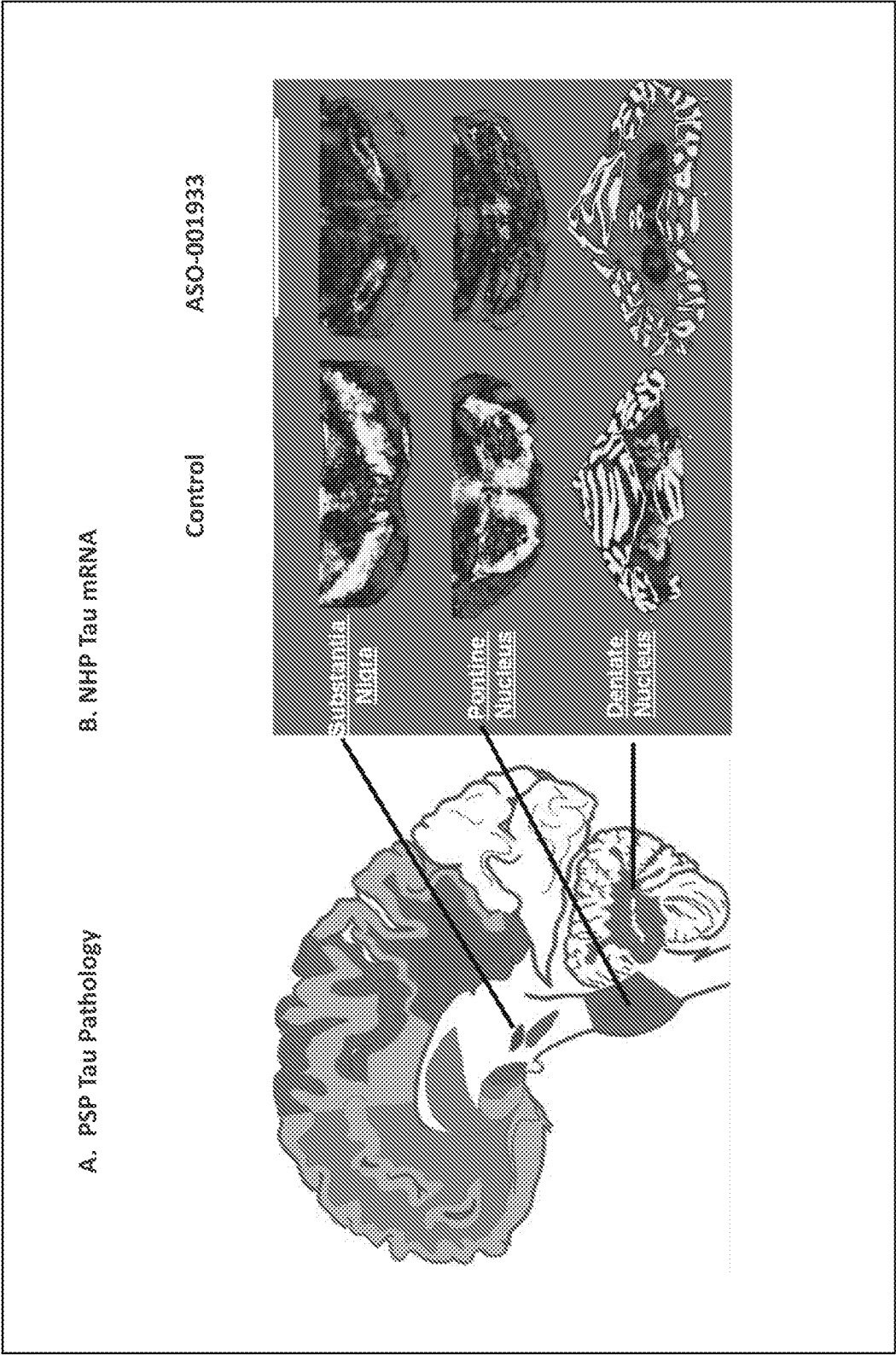


Figure 9

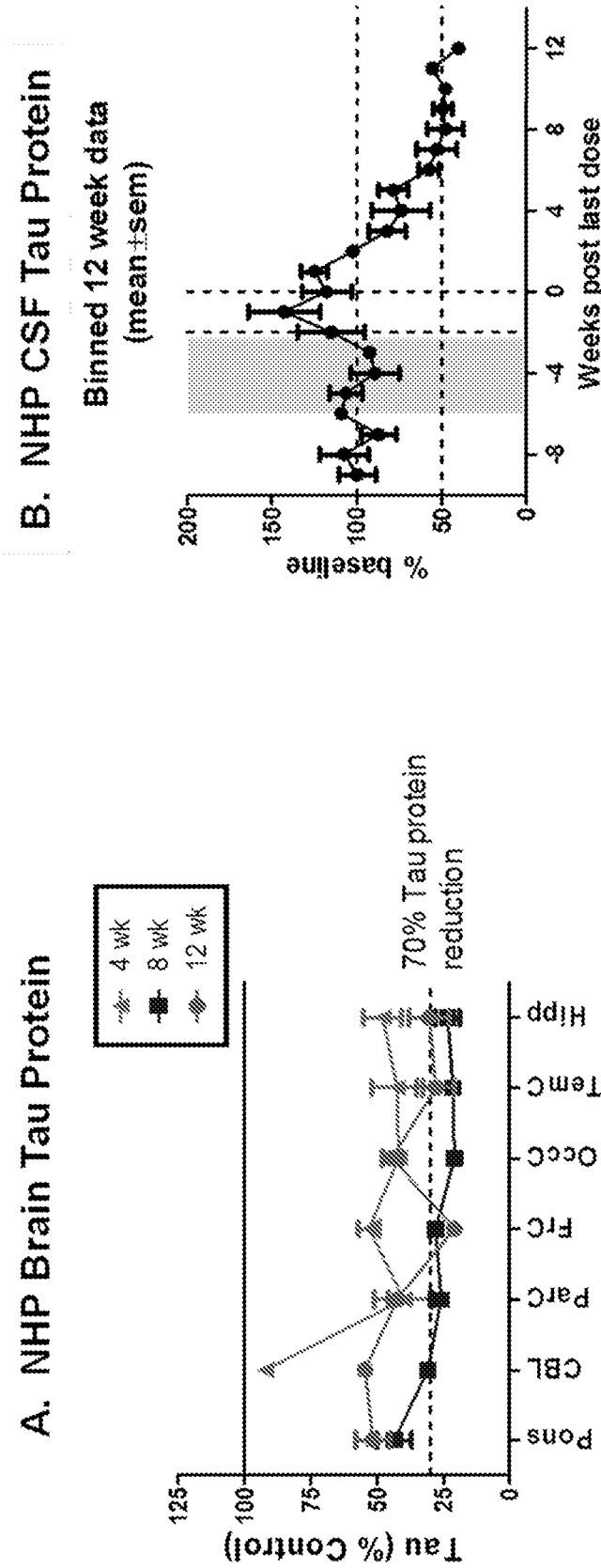


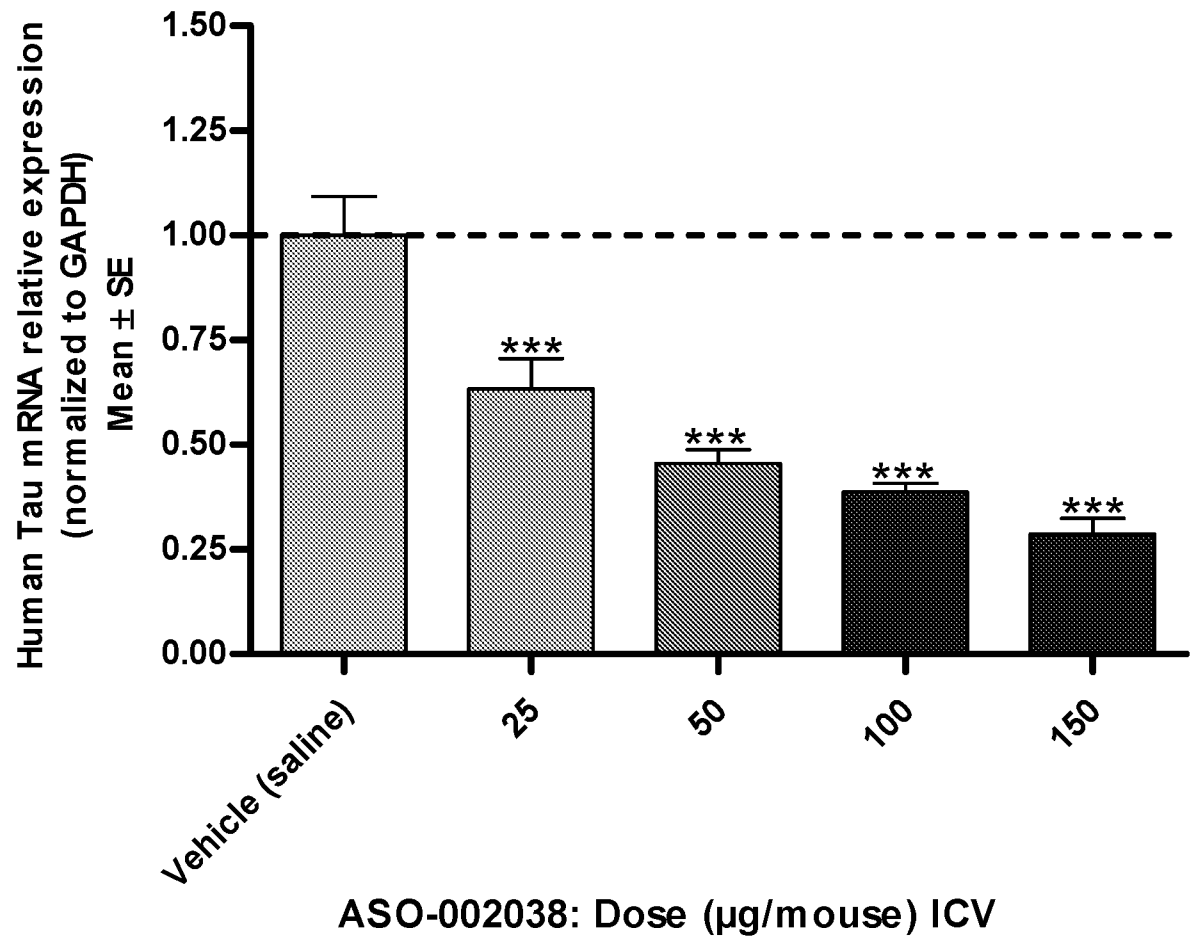
Figure 10A

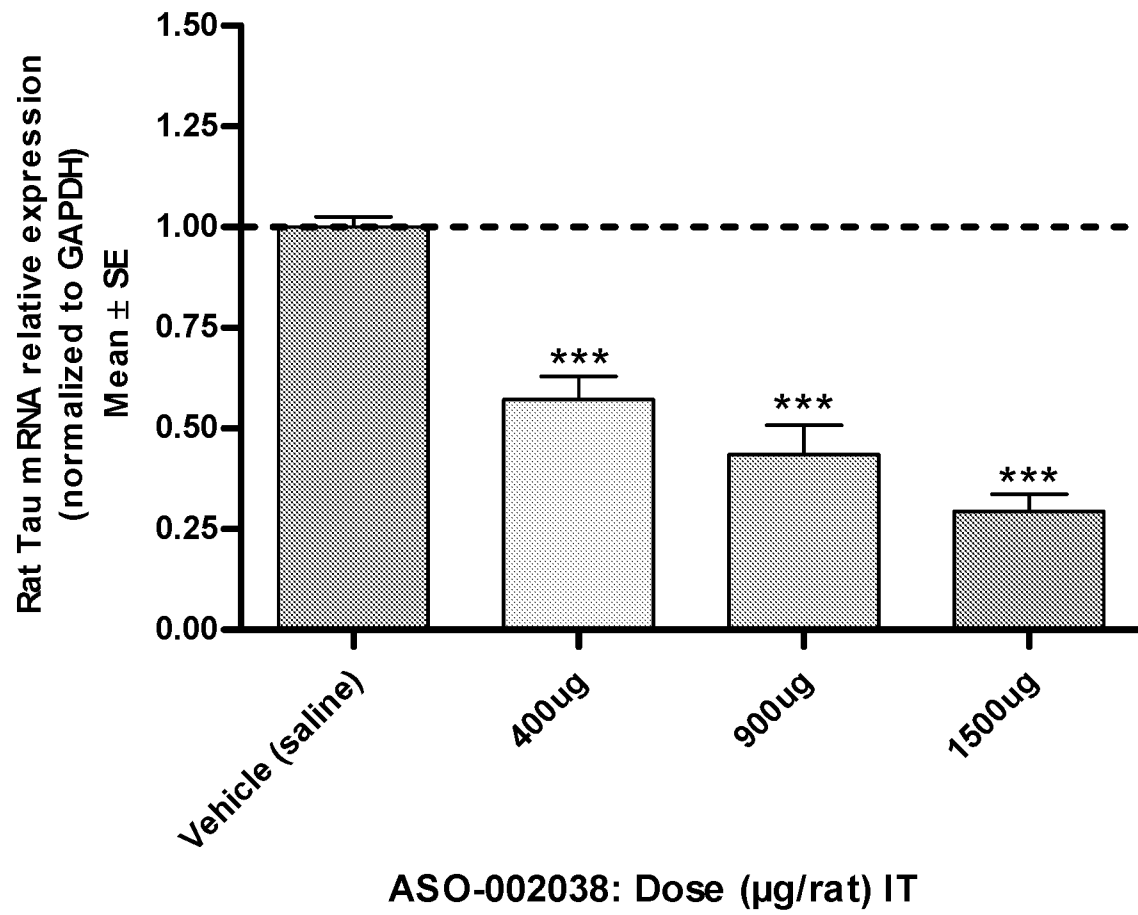
Figure 10B

Figure 11A

ASO No.	SEQ ID NO.	Sequence	tau Human Transcript start (SEQID NO.: 1)	Human Transcript tau end (SEQID NO.: 1)	mRNA start NM_016835	Tau/Tubulin % inhibition
ASO-257283	319	AtTTCaaattcactTTtAC	138884	138903	6722	
ASO-257284	320	AtTTCaaattcactTTtAC	138884	138903	6722	
ASO-002627	321	CcaaattcactTTtAC	138884	138899	6722	
ASO-002677	322	TCcaaattcactTTtAC	138884	138900	6722	
ASO-002670	323	TTCcaaattcactTTtAC	138884	138901	6722	
ASO-002663	324	tTTCcaaattcactTTtAC	138884	138902	6722	
ASO-002635	325	caaattcactTTtAC	138884	138898	6722	
ASO-002643	326	tTTCcaaattcactTTtA	138885	138902	6723	
ASO-002671	327	TCcaaattcactTTtA	138885	138900	6723	
ASO-002664	328	TTCcaaattcactTTtA	138885	138901	6723	
ASO-002626	329	AtTTCcaaattcactTTtA	138885	138903	6723	
ASO-002634	330	AtTTCcaaattcactTTt	138886	138903	6724	
ASO-002642	334	AtTTCcaaattcactTT	138887	138903	6725	
ASO-002649	335	AtTTCcaaattcactT	138888	138903	6726	
ASO-002656	336	AtTTCcaaattcact	138889	138903	6727	

Figure 11B

ASO No. (SEQ ID NO.)	Sequence	tau Human Transcript start (SEQ ID NO.: 1)	Human Transcript tau end (SEQ ID NO.: 1)	Structure
ASO-257283 (319)	AtTTCaaattcactTTtAC	138884	138903	OxyA DNAts OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAAAs DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-257284 (320)	AtTTCaaattcactTTtAC	138884	138903	OxyAs DNAts OxyTs OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAAAs DNAt DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-002627 (321)	CcaaattcactTTtAC	138884	138899	OxyMCs DNACs DNAAAs DNAAAs DNAAAs DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-002677 (322)	TCcaaattcactTTtAC	138884	138900	OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAts DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-002670 (323)	TTCCaaattcactTTtAC	138884	138901	OxyTs OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAAAs DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-002663 (324)	tTTCaaattcactTTtAC	138884	138902	DNAts OxyTs OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAAAs DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-002635 (325)	caaattcactTTtAC	138884	138898	DNACs DNAAAs DNAAAs DNAAAs DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyAs OxyMC
ASO-002643 (326)	tTTCaaattcactTTtA	138885	138902	DNAts OxyTs OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAAAs DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyA
ASO-002671 (327)	TCcaaattcactTTtA	138885	138900	OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAts DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts OxyA
ASO-002664 (328)	TTCCaaattcactTTtA	138885	138901	OxyTs OxyTs OxyMCs DNACs DNAAAs DNAAAs DNAAAs DNAts DNAts DNACs DNAAAs DNACs DNAts OxyTs OxyTs DNAts

Figure 11B (cont.)

				DNAts OxyA
ASO-002626 (329)	AtTTCcaaattcactTTtA	138885	138903	OxyAs DNAts OxyTs OxyTt OxyMCs DNAts DNAts DNAAs DNAts DNAts DNAts DNAts DNAts DNAts OxyTs OxyTs DNAts OxyA
ASO-002634 (330)	AtTTCcaaattcactTTt	138886	138903	OxyAs DNAts OxyTs OxyTt OxyMCs DNAts DNAts DNAAs DNAts DNAts DNAts DNAts DNAts DNAts OxyTs OxyTs DNAt
ASO-002642 (334)	AtTTCcaaattcactTT	138887	138903	OxyAs DNAts OxyTs OxyTt OxyMCs DNAts DNAts DNAAs DNAts DNAts DNAts DNAts DNAts DNAts OxyTs OxyT
ASO-002649 (335)	AtTTCcaaattcactT	138888	138903	OxyAs DNAts OxyTs OxyTt OxyMCs DNAts DNAts DNAAs DNAts DNAts DNAts DNAts DNAts DNAts OxyT
ASO-002656 (336)	AtTTCcaaattcact	138889	138903	OxyAs DNAts OxyTs OxyTt OxyMCs DNAts DNAts DNAAs DNAts DNAts DNAts DNAts DNAts DNAt

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/016657

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/11 C12N15/113 A61K31/712
ADD.

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)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE, EMBL

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Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

20 April 2016

Date of mailing of the international search report

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Bucka, Alexander

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PCT/US2016/016657

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