[0006] In general, difficulties in the use of RNAi technology in the past have included off-target effects related to the use of guide strands insufficiently tailored to affect specific genes, delivery to multiple organ systems where gene expression of the target gene may be desirable and having the capability to target oligonucleotides to organ systems other than the liver where the characteristics of hepatocytes assist in the uptake and effectiveness of RNAi technology.

[0007] In terms of pathologies of the Central Nervous System ("CNS") most pharmacotherapies currently being used for treatment of neurodegenerative or inflammatory CNS disorders target molecules that are localized downstream in the pathogenic cascade. Therefore, their effects are often not specific and are moderate or simply ineffective with regard to disease modulation. Other approaches that may add to the medical arsenal are those that focus on different methods of modulating or controlling a disease. Among these innovative therapeutic strategies is the 'silencing' of genes that cause or directly contribute to disease phenotypes using RNAi technologies. The difficulties in using this therapeutic avenue have been identifying specific candidate genes, specific targeting to the CNS, durability of therapeutic effect and the exit from the CNS of RNAi modalities that could affect other tissues.

[0008] The aldehyde dehydrogenase-2 (ALDH2) gene encodes an important biologically active enzyme, ALDH2. ALDH2 participates in the metabolism and detoxification of aldehyde and metabolizes short-chain aliphatic aldehydes and converted acetaldehyde into acetate it is active in the human liver. ALDH2 has been shown involved in the metabolism of other biogenic aldehydes, such as 4-hydroxynonenal, 3,4-dihydroxyphenylacetaldehyde, and 3,4-dihydroxyphenylglycoaldehyde. Recent studies have indicated that ALDH2 is also expressed in the CNS where it exerts protective effects on the cardio-cerebral vascular system and central nervous system. Single nucleotide polymorphisms (SNPs) of the ALDH2 gene have been reported to be associated with the risks for several neurological diseases, such as neurodegenerative diseases, cognitive disorders, and anxiety disorders. Removing or inhibiting the ALDH2 gene in the CNS prevents or limits the biological activity of the active enzyme and is relatively easily measured.

SUMMARY

[0009] Aspects of the disclosure relate to oligonucleotides and related methods for treating a neurological disease in a subject. In some examples, potent RNAi oligonucleotides are provided for their selective activity in the CNS. In the present disclosure the oligonucleotides administered into the CNS are effective at delivering an ALDH2 targeting guide strand that loads into the RISC complex and that thereafter is effective in the inhibition of ALDH2 expression in the central nervous system of a subject *via* the cleavage of ALDH2 mRNAs. In some examples, RNAi oligonucleotides provided herein target key regions of ALDH2 mRNA (referred to as hotspots) that are particularly amenable to targeting using such oligonucleotide-based approaches (*see* Table 5). In some examples, RNAi oligonucleotides provided herein incorporate modified phosphates, nicked tetraloop structures,

and/or other modifications that improve activity, bioavailability and/or minimize the extent of enzymatic degradation after *in vivo* administration to the central nervous system. The ALDH2 gene targeting sequence, according to the present disclosure, could be replaced with a guide strand directed to a gene sequence of interest in a fashion that would allow the specific degradation of mRNA in the CNS and thereby degrade or inhibit the production of a protein of interest. Where this protein is a contributor to gain of function pathology – the negative aspects of the pathology are reduced or eliminated while the RISC complex remains active in cleaving the target mRNA. Other oligonucleotides of the current disclosure can also be put into to the CNS to modulate or inhibit the expression of specific target genes in a therapeutically meaningful way.

[0010] Some examples of the present disclosure provide methods of reducing expression of ALDH2 in a subject, the method comprising administering to the cerebrospinal fluid of the subject an oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length, wherein the antisense strand has a region of complementarity to a target sequence of ALDH2 as set forth in any one of SEQ ID NOs: 601-607, wherein the region of complementarity is at least 12 contiguous nucleotides in length. Some aspects of the present disclosure provide a pharmaceutical composition comprising an oligonucleotide of the disclosure, and a pharmaceutically acceptable carrier. Some aspects of the present disclosure provide a use of an oligonucleotide or a pharmaceutical composition comprising the oligonucleotide in the manufacture of a medicament for reducing expression of ALDH2 in a subject wherein the oligonucleotide comprises an antisense strand of 15 to 30 nucleotides in length and a sense strand of 15 to 40 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand, and wherein the antisense strand has a region of complementarity to a target sequence of ALDH2 as set forth in any one of SEQ ID NOs: 601-607, wherein the region of complementarity is at least 12 contiguous nucleotides in length. In some examples, the region of complementarity is fully complementary to the target sequence of ALDH2. In some examples, the antisense strand is 19 to 27 nucleotides in length.

[0011] In some examples, the oligonucleotide further comprises a sense strand of 15 to 40 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand. In some examples, the sense strand is 19 to 40 nucleotides in length.

[0012] In some examples, the duplex region is at least 12 nucleotides in length. In some examples, the region of complementarity to ALDH2 is at least 13 contiguous nucleotides in length.

[0013] In some examples, the antisense strand comprises a sequence as set forth in any one of SEQ ID NOs: 591-600. In some examples, the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 581-590, 608, and 609. In some examples, the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 591-600. In some examples, the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 581-590, 608, and 609.

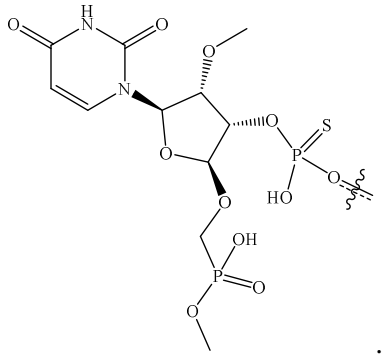
[0014] In some examples, the oligonucleotide comprises at least one modified nucleotide. In some examples, the modified nucleotide comprises a 2′-modification. In some examples, the 2′-modification is a modification selected from: 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, 2′- aminodiethoxymethanol, 2′- adem, and 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid. In some examples, all of the nucleotides of the oligonucleotide are modified.

[0015] In some examples, the oligonucleotide comprises at least one modified internucleotide linkage. In some examples, the at least one modified internucleotide linkage is a phosphorothioate linkage.

[0016] In some examples, the oligonucleotide comprises a phosphorothioate linkage between one or more of: positions 1 and 2 of the sense strand, positions 1 and 2 of the antisense strand, positions 2 and 3 of the antisense strand, positions 3 and 4 of the antisense strand, positions 20 and 21 of the antisense strand, and/or positions 21 and 22 of the antisense strand. In some examples, the oligonucleotide has a phosphorothioate linkage between each of: positions 1 and 2 of the sense strand, positions 1 and 2 of the antisense strand, positions 2 and 3 of the antisense strand, positions 20 and 21 of the antisense strand, and positions 21 and 22 of the antisense strand.

[0017] In some examples, the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog. In some examples, the phosphate analog is oxymethylphosphonate, vinylphosphonate, or malonylphosphonate.

[0018] In some examples, a uridine present at the first position of an antisense strand comprises a phosphate analog. In some examples, the oligonucleotide comprises the following structure at position 1 of the antisense strand:

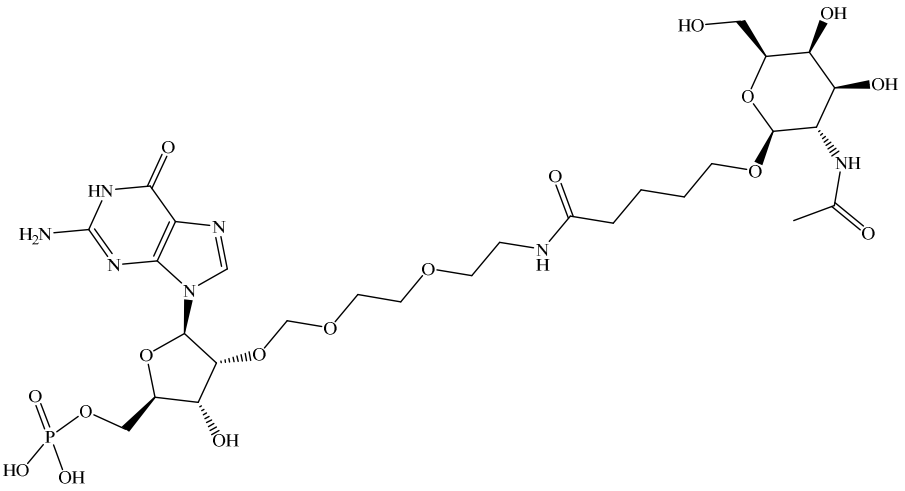


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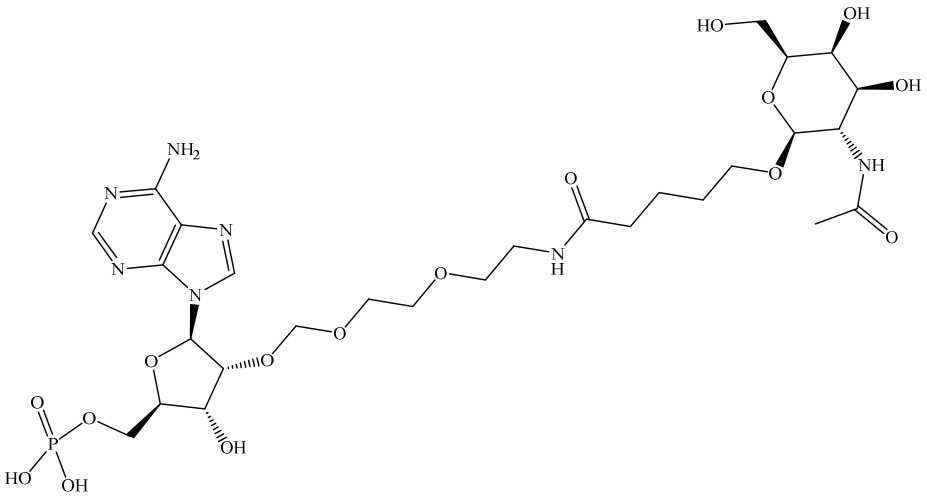
[0019] In some examples, the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length. In some examples, L is a tetraloop. In some examples, L is 4 nucleotides in length. In some examples, L comprises a sequence set forth as GAAA.

[0020] In some examples, one or more of the nucleotides of the GAAA sequence at positions 27-30 on the sense strand is conjugated to a monovalent GalNAc moiety. In some examples, each of the

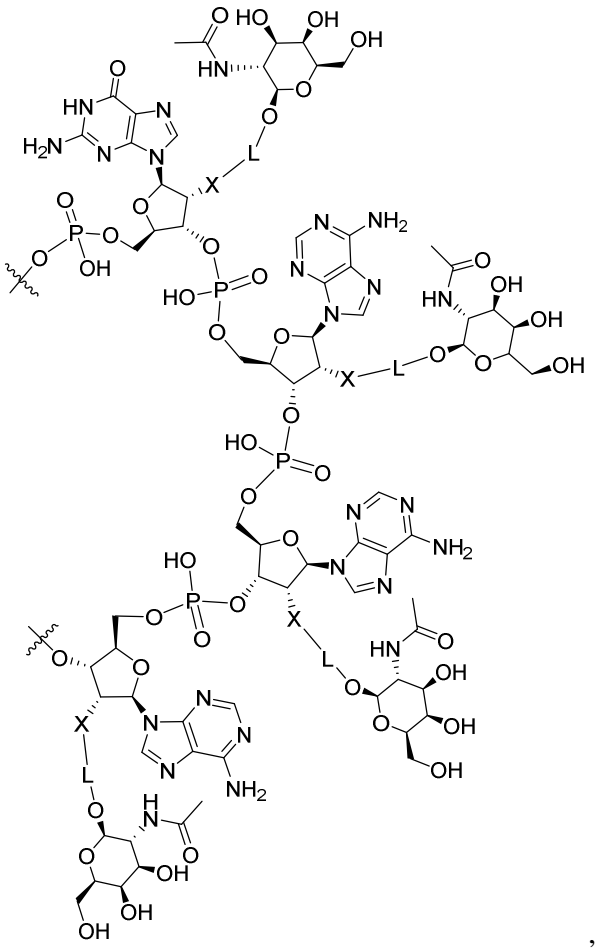
nucleotides of the GAAA sequence at positions 27-30 on the sense strand is conjugated to a monovalent GalNAc moiety. In some examples, each of A of the GAAA sequence (at positions 28-30) on the sense strand is conjugated to a monovalent GalNAc moiety. In some examples, an oligonucleotide herein comprises a monovalent GalNAc attached to a Guanidine nucleotide, referred to as [ademG-GalNAc] or 2'-aminodiethoxymethanol-Guanidine-GalNAc, as depicted below:



[0021] In some examples, an oligonucleotide herein comprises a monovalent GalNAc attached to an adenine nucleotide, referred to as [ademA-GalNAc] or 2'-aminodiethoxymethanol-Adenine-GalNAc, as depicted below.



[0022] In some examples, the GAAA motif at positions 27-30 on the sense strand comprises the structure:

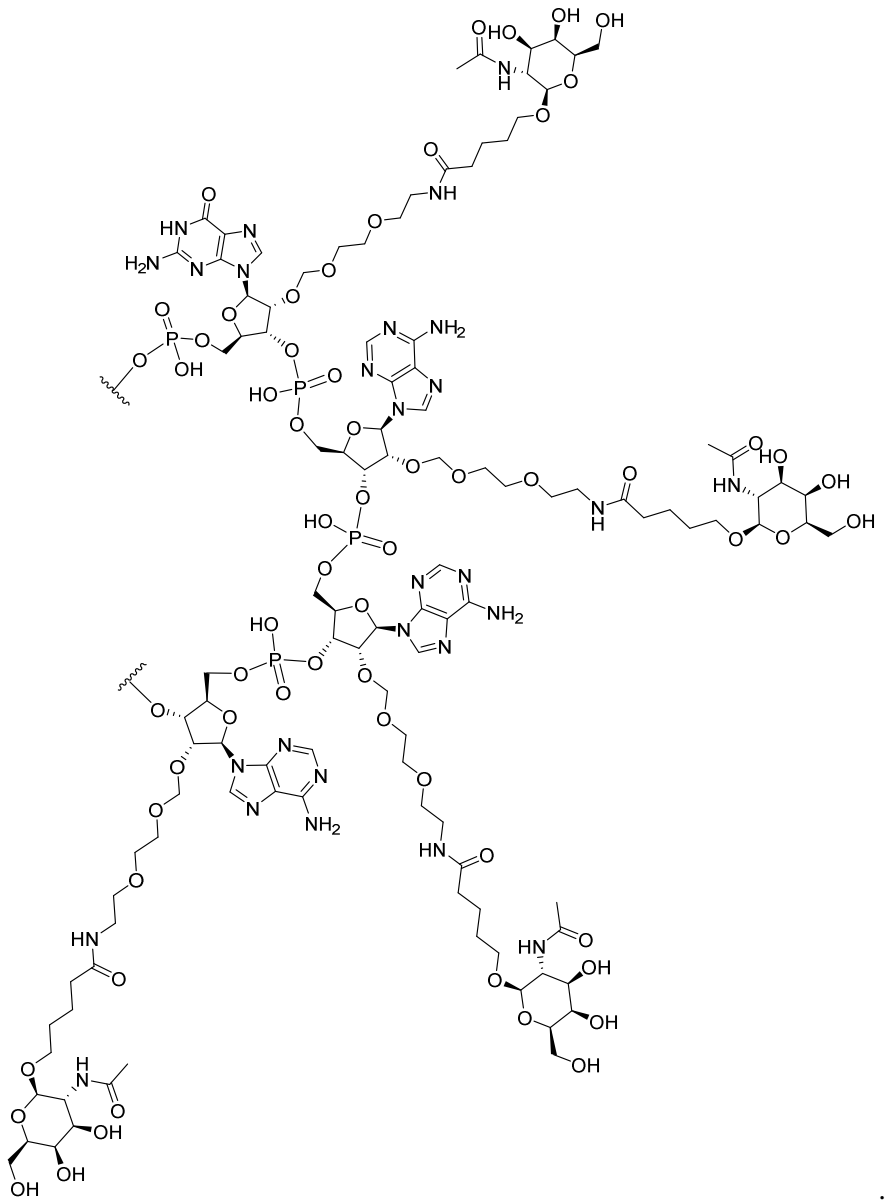


wherein:

L represents a bond, click chemistry handle, or a linker of 1 to 20, inclusive, consecutive, covalently bonded atoms in length, selected from the group consisting of substituted and unsubstituted alkylene, substituted and unsubstituted alkenylene, substituted and unsubstituted alkynylene, substituted and unsubstituted heteroalkylene, substituted and unsubstituted heteroalkenylene, substituted and unsubstituted heteroalkynylene, and combinations thereof; and X is O, S, or N.

[0023] In some examples, L is an acetal linker. In some examples, X is O.

[0024] In some examples, the GAAA sequence at positions 27-30 on the sense strand comprises the structure:



[0025] In some examples, each of the A in the GAAA sequence is conjugated to a GalNAc moiety (e.g., at positions 28-30 on the sense strand). In some examples, the GalNAc moiety conjugated to each of A has the structure illustrated above, except that G is unmodified or has a 2' modification on the sugar moiety. In some examples, the G in the GAAA sequence comprises a 2′-O-methyl modification (e.g., 2'-O-methyl or 2′-O-methoxyethyl), and each of A in the GAAA sequence is conjugated to a GalNAc moiety, such as in portions of the structures illustrated above.

[0026] In some examples, the G in the GAAA sequence comprises a 2′-OH. In some examples, each of the nucleotides in the GAAA sequence comprises a 2′-O-methyl modification. In some examples, each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification. In some examples, each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and the G in the GAAA sequence comprises a 2′-O-

methyl modification. In some examples, each of the A in the GAAA sequence comprises a 2′- adem modification and the G in the GAAA sequence comprises a 2′-O-methyl modification.

[0027] In some examples, the antisense strand and the sense strand are not covalently linked.

[0028] In some examples, the oligonucleotide is administered intrathecally, intraventricularly, intracavitary, or interstitially. In some examples, the oligonucleotide is administered via injection or infusion.

[0029] In some examples, the subject has a neurological disorder. In some examples, the neurological disorder is selected from: neurodegenerative diseases, cognitive disorders, and anxiety disorders.

[0030] In some examples, the method of reducing expression of ALDH2 in a subject comprises administering to the cerebrospinal fluid of the subject an oligonucleotide comprising an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ALDH2,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length,

and wherein the antisense strand and the sense strand form a duplex structure of at least 12 nucleotides in length but are not covalently linked.

[0031] In some examples, the method of reducing expression of ALDH2 in a subject comprises administering to the cerebrospinal fluid of the subject an oligonucleotide comprising an antisense strand and a sense strand that are not covalently linked,

wherein the antisense strand comprises a sequence as set forth in SEQ ID NO: 595 and the sense strand comprises a sequence as set forth in SEQ ID NO: 585,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L is a tetraloop comprising a sequence set forth as GAAA, and wherein the GAAA sequence comprises a structure selected from the group consisting of:

(i) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(ii) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-OH;

(iii) each of the nucleotide in the GAAA sequence comprises a 2′-O-methyl modification;

(iv) each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(v) each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and

the G in the GAAA sequence comprises a 2′-O-methyl modification; and

(vi) each of the A in the GAAA sequence comprises a 2′- aminodiethoxymethanol modification and the G in the GAAA sequence comprises a 2′-O-methyl modification.

[0032] In some examples, the method of reducing expression of ALDH2 in a subject comprises administering to the cerebrospinal fluid of the subject an oligonucleotide comprising an antisense strand and a sense strand that are not covalently linked, wherein the antisense strand comprises a sequence as set forth in SEQ ID NO: 595 and the sense strand comprises a sequence as set forth in SEQ ID NO: 609. In some aspects, the disclosure provides a use of an oligonucleotide or a composition comprising an oligonucleotide in the manufacture of a medicament for reducing expression of ALDH2 in a subject wherein the oligonucleotide comprises an antisense strand and a sense strand that are not covalently linked, wherein the antisense strand comprises a sequence as set forth in SEQ ID NO: 595 and the sense strand comprises a sequence as set forth in SEQ ID NO: 585, or wherein the antisense strand comprises a sequence as set forth in SEQ ID NO: 595 and the sense strand comprises a sequence as set forth in SEQ ID NO: 609.

[0033] In some examples, the oligonucleotide reduces expression detectable in somatosensory cortex, hippocampus, frontal cortex, striatum, hypothalamus, cerebellum, and/or spinal cord.

[0034] Other examples of the present disclosure provide methods of reducing expression of a gene of interest in a subject, the method comprising administering to the cerebrospinal fluid of the subject an oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length, wherein the antisense strand has a region of complementarity to a target sequence of said gene of interest that expresses in the CNS, wherein the region of complementarity is at least 12 contiguous nucleotides in length.

[0035] In some examples, the gene of interest is selected from the group consisting of ALDH2, Ataxin-1, Ataxin-3, APP, BACE1, DYT1, and SOD1.

[0036] In some examples, the oligonucleotide reduces expression detectable in somatosensory cortex, hippocampus, frontal cortex, striatum, hypothalamus, cerebellum, and/or spinal cord.

[0037] In some examples, the oligonucleotide further comprising elements that are degraded by nucleases outside the CNS such that said nucleotide is no longer capable of reducing expression of a gene of interest in a subject in tissues outside the CNS.

[0038] In some examples, the oligonucleotide further comprises modifications such that it cannot easily exit the CNS.

[0039] Other examples of the present disclosure provide methods of treating a neurological disorder, the method comprising administering to the cerebrospinal fluid of a subject in need thereof an oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length, wherein the

antisense strand has a region of complementarity to a target sequence of ALDH2 as set forth in any one of SEQ ID NOs: 601-607, wherein the region of complementarity is at least 12 contiguous nucleotides in length.

[0040] In some examples, the method comprises administering to the cerebrospinal fluid of a subject in need thereof an oligonucleotide comprising an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ALDH2,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length,

and wherein the antisense strand and the sense strand form a duplex structure of at least 12 nucleotides in length but are not covalently linked.

[0041] In some examples, the neurological disorder is a neurodegenerative disease. In some examples, the neurological disorder is an anxiety disorder.

[0042] In some examples, the oligonucleotide is administered intrathecally, intraventricularly, intracavitary, or interstitially. In some examples, the oligonucleotide is administered via injection or infusion.

[0043] In some examples, the oligonucleotide reduces expression detectable in somatosensory cortex, hippocampus, frontal cortex, striatum, hypothalamus, cerebellum, and/or spinal cord.

[0044] Other aspects of the present disclosure provide oligonucleotides comprising an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ALDH2,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L is a tetraloop and comprises a sequence set forth as GAAA, wherein the GAAA sequence comprises a structure selected from the group consisting of:

(i) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(ii) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-OH;

(iii) each of the nucleotide in the GAAA sequence comprises a 2′-O-methyl modification;

(iv) each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(v) each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and the G in the GAAA sequence comprises a 2′-O-methyl modification; and

(vi) each of the A in the GAAA sequence comprises a 2′- adem modification and the G in the GAAA sequence comprises a 2′-O-methyl modification,

and wherein the antisense strand and the sense strand form a duplex structure of at least 12 nucleotides in length but are not covalently linked.

[0045] In some examples, the antisense strand comprises a sequence set forth in any one of SEQ ID NOs: 591-600. In some examples, the sense strand comprises a sequence set forth in any one of SEQ ID NOs: 581-590. Compositions comprising these oligonucleotides and an excipient are provided. In some examples, a method of reducing expression ALDH2 in a subject comprises administering the composition to the cerebrospinal fluid of the subject. In some examples, a method of treating a neurological disease in a subject in need thereof comprises administering the composition to the cerebrospinal fluid of the subject.

[0046] Other examples of the present disclosure provide methods of reducing expression of a target gene in a subject, the method comprising administering an oligonucleotide to the cerebrospinal fluid of the subject, wherein the oligonucleotide comprises an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to the target gene,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length,

and wherein the antisense strand and the sense strand form a duplex structure of at least 12 nucleotides in length but are not covalently linked. Other aspects of the present disclosure provide a use of an oligonucleotide in the manufacture of a medicament for reducing expression of a target gene in a subject, wherein the use comprises that the oligonucleotide is to be administered to the cerebrospinal fluid of the subject, wherein the oligonucleotide comprises an antisense strand and a sense strand, wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to the target gene of interest that is expressed in the CNS, wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length, and wherein the antisense strand and the sense strand form a duplex structure of at least 12 nucleotides in length but are not

covalently linked; and wherein L is a tetraloop; and wherein L comprises a sequence set forth as GAAA.

[0047] In some examples, Lis a tetraloop. In some examples, L is 4 nucleotides in length. In some examples, L comprises a sequence set forth as GAAA. In some examples, each of the A in GAAA sequence is conjugated to a GalNAc moiety. In some examples, the G in the GAAA sequence comprises a 2′-O-methyl modification. In some examples, the G in the GAAA sequence comprises a 2′-OH. In some examples, each of the nucleotide in the GAAA sequence comprises a 2′-O-methyl modification. In some examples, each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification. In some examples, each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and the G in the GAAA sequence comprises a 2′-O-methyl modification. In some examples, each of the A in the GAAA sequence comprises a 2′- adem and the G in the GAAA sequence comprises a 2′-O-methyl modification.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain embodiments, and together with the written description, serve to provide non-limiting examples of certain aspects of the compositions and methods disclosed herein.

[0049] FIG. 1 shows the regions of the brain for intraventricular (ICV) administration of RNAi oligonucleotides of interest to a CD-1 mouse (25 g female).

CLAIMS

What is claimed is:

1. An oligonucleotide comprising an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ALDH2,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L is a tetraloop and comprises a sequence set forth as GAAA, wherein the GAAA sequence comprises a structure selected from the group consisting of:

(i) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(ii) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-OH;

(iii) each of the nucleotide in the GAAA sequence comprises a 2′-O-methyl modification;

(iv) each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(v) each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and the G in the GAAA sequence comprises a 2′-O-methyl modification; and

(vi) each of the A in the GAAA sequence comprises a 2′- adem modification and the G in the GAAA sequence comprises a 2′-O-methyl modification,

and wherein the antisense strand and the sense strand form a duplex structure of at least 12 nucleotides in length but are not covalently linked.

2. The oligonucleotide of claim 1, wherein the antisense strand comprises a sequence set forth in any one of SEQ ID NOs: 591-600, and wherein the sense strand comprises a sequence set forth in any one of SEQ ID NOs: 581-590.

3. A pharmaceutical composition comprising an oligonucleotide of claims 1 or 2, and a pharmaceutically acceptable carrier.

4. Use of an oligonucleotide or a pharmaceutical composition comprising the oligonucleotide in the manufacture of a medicament for reducing expression of ALDH2 in a subject wherein the oligonucleotide comprises an antisense strand of 15 to 30 nucleotides in length and a sense strand of 15 to 40 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand, and wherein the antisense strand has a region of complementarity to a target sequence of ALDH2 as set forth in any one of SEQ ID NOs: 601-607, wherein the region of complementarity is at least 12 contiguous nucleotides in length.

5. The use of claim 4, wherein the region of complementarity is fully complementary to the target sequence of ALDH2.

6. The use of claim 4 or 5, wherein the antisense strand is 19 to 27 nucleotides in length, and comprises a sequence as set forth in any one of SEQ ID NOs: 591-600.

7. The use of any one of claims 4 to 6, wherein the oligonucleotide comprises at least one modified nucleotide, wherein the modified nucleotide comprises a 2′-modification, and wherein the 2′-modification is a modification selected from: 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, 2′-adem, 2'- aminodiethoxymethanol, and 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid.

8. The use of any one of claims 4 to 7, wherein

a. the oligonucleotide comprises at least one modified internucleotide linkage, and wherein the at least one modified internucleotide linkage is a phosphorothioate linkage; and

b. the antisense strand comprises a phosphate analog at the 4′-carbon of the sugar of the 5′-nucleotide; and wherein the phosphate analog is oxymethylphosphonate, vinylphosphonate, or malonylphosphonate.

9. The use of any one of claims 4 to 8, wherein the sense strand is 19 to 40 nucleotides in length; and wherein the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 581-590, 608, and 609.

10. The use of any one of claims 4 to 9, wherein the region of complementarity to ALDH2 is at least 13 contiguous nucleotides in length.

11. The use of any one of claims 4 to 10, wherein the sense strand comprises at its 3′-end a stem-loop sequence set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length.

12. The use of claim 11, wherein L is a tetraloop, wherein L is 4 nucleotides in length, and wherein L comprises a sequence set forth as GAAA, and wherein at least one nucleotide in the GAAA sequence is conjugated to a GalNAc moiety.

13. The use of claim 12, wherein the GAAA sequence comprises a structure selected from the following:

(i) each of the A in GAAA sequence is conjugated to a GalNAc moiety;

(ii) the G in the GAAA sequence comprises a 2′-O-methyl modification;

(iii) the G in the GAAA sequence comprises a 2′-OH;

(iv) each of the nucleotide in the GAAA sequence comprises a 2′-O-methyl modification;

(v) each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(vi) each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and the G in the GAAA sequence comprises a 2′-O-methyl modification; and

(vii) each of the A in the GAAA sequence comprises a 2′- adem and the G in the GAAA sequence comprises a 2′-O-methyl modification.

14. The method of any one of claims 4 to 13, wherein the subject has a neurological disorder; wherein the neurological disorder is selected from: neurodegenerative diseases, cognitive disorders, and anxiety disorders.

15. Use of an oligonucleotide or a composition comprising an oligonucleotide in the manufacture of a medicament for reducing expression of ALDH2 in a subject wherein the oligonucleotide comprises an antisense strand and a sense strand that are not covalently linked,

wherein the antisense strand comprises a sequence as set forth in SEQ ID NO: 595 and the sense strand comprises a sequence as set forth in SEQ ID NO: 585, or wherein the antisense strand comprises a sequence as set forth in SEQ ID NO: 595 and the sense strand comprises a sequence as set forth in SEQ ID NO: 609

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L is a tetraloop comprising a sequence set forth as GAAA, and wherein the GAAA sequence comprises a structure selected from the group consisting of:

(i) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(ii) each of the A in GAAA sequence is conjugated to a GalNAc moiety, and the G in the GAAA sequence comprises a 2′-OH;

(iii) each of the nucleotide in the GAAA sequence comprises a 2′-O-methyl modification;

(iv) each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(v) each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and the G in the GAAA sequence comprises a 2′-O-methyl modification; and

(vi) each of the A in the GAAA sequence comprises a 2′- adem modification and the G in the GAAA sequence comprises a 2′-O-methyl modification.

16. The use of any one of claims 4 to 15, wherein the oligonucleotide reduces expression of ALDH2 that is detectable in somatosensory cortex, hippocampus, frontal cortex, striatum, hypothalamus, cerebellum, and/or spinal cord.

17. The use of any one of claims 4-16 wherein reduction in expression of ALDH2 is for treating a neurological disorder associated with ALDH2 expression, wherein the use comprises that the medicament is to be administered to the cerebrospinal fluid of a subject in need thereof.

18. Use of an oligonucleotide in the manufacture of a medicament for reducing expression of a target gene in a subject, wherein the use comprises that the oligonucleotide is to be administered to the cerebrospinal fluid of the subject, wherein the oligonucleotide comprises an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to the target gene of interest that is expressed in the CNS,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length,

and wherein the antisense strand and the sense strand form a duplex structure of at least 12 nucleotides in length but are not covalently linked; and wherein L is a tetraloop; and wherein L comprises a sequence set forth as GAAA.

19. The use of claim 18, wherein the GAAA sequence comprises a structure selected from the following:

(i) each of the A in GAAA sequence is conjugated to a GalNAc moiety;

(ii) the G in the GAAA sequence comprises a 2′-O-methyl modification;

(iii) the G in the GAAA sequence comprises a 2′-OH;

(iv) each of the nucleotide in the GAAA sequence comprises a 2′-O-methyl modification;

(v) each of the A in the GAAA sequence comprises a 2′-OH and the G in the GAAA sequence comprises a 2′-O-methyl modification;

(vi) each of the A in the GAAA sequence comprises a 2′-O-methoxyethyl modification and the G in the GAAA sequence comprises a 2′-O-methyl modification; and

(vii) each of the A in the GAAA sequence comprises a 2′- adem and the G in the GAAA sequence comprises a 2′-O-methyl modification.

20. The use of any one of claims 17 to 19, wherein the target gene is selected from the group consisting of ALDH2, Ataxin-1, Ataxin-3, APP, BACE1, DYT1, and SOD1; and wherein the oligonucleotide reduces expression of the target gene in somatosensory cortex, hippocampus, frontal cortex, striatum, hypothalamus, cerebellum, and/or spinal cord.

21. The use of any one of claims 17 to 20, wherein the oligonucleotide further comprises elements that are degraded by nucleases outside the CNS such that said nucleotide is no longer capable of reducing expression of a gene of interest in a subject in tissues outside the CNS; and wherein the oligonucleotide further comprises modifications such that it cannot easily exit the CNS.