

ANTISENSE OLIGOMERS FOR TREATMENT OF CONDITIONS AND DISEASES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/945,048 filed December 6, 2019, and U.S. Provisional Application No. 62/993,971, filed March 24, 2020, each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 28, 2021, is named 47991-728_601_SL.txt and is 1,169,854 bytes in size.

BACKGROUND

[0002] Nervous system disorders are often associated with channelopathy, characterized by the disturbed function of ion channels that mediate neuronal excitability, neuronal interactions, and brain functions at large. Mutations in the *SCN1A* gene, which is part of the *SCN1A-SCN2A-SCN3A* gene cluster that encodes alpha-pore forming subunits of the neuronal voltage gated sodium channel, can result in expression of Nav1.1 protein (also termed as “Nav1.1”) with reduced functions as compared to a wild-type Nav1.1 protein, reduced expression of Nav1.1, or both. Mutations in *SCN1A* gene are associated with development of disease number of diseases and conditions, such as Dravet Syndrome (DS) (Miller, et al., 1993-2015, GeneReviews, Eds. Pagon RA, et al. Seattle (WA): University of Washington, Seattle, Bookshelf ID: NBK1318, and Mulley, et al., 2005, Hum. Mutat. 25: 535-542).

SUMMARY

[0003] Alternative splicing events in *SCN1A* gene can lead to non-productive mRNA transcripts which in turn can lead to aberrant protein expression, and therapeutic agents which can target the alternative splicing events in *SCN1A* gene can modulate the expression level of functional proteins in Dravet Syndrome patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition caused by *SCN1A*, *SCN8A* or *SCN5A* protein deficiency.

[0004] Selecting the right dose, formulation, dosing regimen and patient population for a drug is a crucial step in the development of pharmaceutical drugs. For example, without adequate information on dosage, it is not possible for doctors to prescribe a drug to patients. For example, if a dose or dose range cannot be identified that allows safe and predictable administration, the drug cannot be a medically useful or commercially viable pharmaceutical product. Therefore, determining the correct drug dosage is a key question that needs to be addressed in clinical practice. The discovery of therapeutically effective dosages and dosage regimens of a drug needs to balance patient compliance, therapeutic efficacy and side effects of the drug; which requires substantial skills. For example, suitable dosages and dosage regimens may be discovered through a clinical trial that forms part of the approval process, requires substantial input of intellectual and financial resources of different parties, and is not within the routine of a medical practitioner. For example, patient compliance can be crucial for optimal treatment of various conditions. The more doses

required or the more difficult the treatment plan, the less likely a patient is to comply; although medical agents have the ability to enhance patients' quality of life (QOL), they can only do so if they are used correctly. Thus, it is clear that selecting the right dose, formulation, dosing regimen and patient population for a drug is complex and unpredictable. Structurally similar compounds significantly differ in their

solubility, toxicity, activity, stability, and pharmacological properties. Also, there are significant physiological differences between animal models and human subjects. Therefore, translating pre-clinical information into clinically effective therapy is an unpredictable and challenging task.

[0005] Provided herein are suitable dosages, formulations, dosing regimens and patient populations for treating or reducing the likelihood of developing a disease or condition characterized by a reduced expression or function of Nav1.1 protein in a human subject in need thereof.

[0005a] In some aspects, provided herein is a use of an antisense oligomer (ASO) for the manufacture of a medicament for treating or reducing the likelihood of developing a disease or condition characterized by a reduced expression or function of Nav1.1 protein in a human subject in need thereof, wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 21-67, 210-256 or 304-1099, and wherein the medicament is to be administered at a dose of from about 0.5 milligrams to about 500 milligrams of the ASO.

[0005b] In some aspects, provided herein is a pharmaceutical formulation comprising:

- (a) an antisense oligomer (ASO), wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 21-67, 210-256 or 304-1099; and
- (b) a pharmaceutically acceptable diluent;

[0006] wherein the ASO is present in the pharmaceutical formulation at a concentration of from 0.1 mg/mL to 200 mg/mL. In some aspects, provided herein is a method of treating or reducing the likelihood of developing a disease or condition characterized by a reduced expression or function of Nav1.1 protein in a human subject in need thereof, the method comprising administering to the human subject a pharmaceutical composition comprising an antisense oligomer (ASO) at a first dose of about 0.1, 0.5, 1, 2.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, 57.5, 60, 62.5, 65, 67.5, 70, 72.5, 75, 77.5, 80, 82.5, 85, 87.5, 90, 92.5, 95, 97.5, 100, 102.5, 105, 107.5, 110, 112.5, 115, 117.5, 120, 122.5, 125, 127.5, 130, 132.5, 135, 137.5, 140, 142.5, 145, 147.5, 150, 152.5, 155, 157.5, 160, 162.5, 165, 167.5, 170, 172.5, 175, 177.5, 180, 182.5, 185, 187.5, 190, 192.5, 195, 197.5, 200, 202.5, 205, 207.5, 210, 212.5, 215, 217.5, 220, 222.5, 225, 227.5, 230, 232.5, 235, 237.5, 240, 242.5, 245, 247.5, or 250 mg, wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 21-67, 210-256 or 304-1099, thereby treating or reducing the likelihood of developing the disease or condition in the human subject.

[0007] In some aspects, provided herein is a use of an antisense oligomer (ASO) for the manufacture of a medicament for treating or preventing a disease or condition characterized by a reduced expression or function of Nav1.1 protein in a human subject in need thereof, wherein the medicament is administered at a first dose of about 0.1, 0.5, 1, 2.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, 57.5, 60, 62.5, 65, 67.5, 70, 72.5, 75, 77.5, 80, 82.5, 85, 87.5, 90, 92.5, 95, 97.5, 100, 102.5, 105, 107.5, 110, 112.5, 115, 117.5, 120, 122.5, 125, 127.5, 130, 132.5, 135, 137.5, 140, 142.5, 145, 147.5, 150, 152.5, 155, 157.5, 160, 162.5, 165, 167.5, 170, 172.5, 175, 177.5, 180, 182.5, 185, 187.5, 190,

192.5, 195, 197.5, 200, 202.5, 205, 207.5, 210, 212.5, 215, 217.5, 220, 222.5, 225, 227.5, 230, 232.5, 235, 237.5, 240, 242.5, 245, 247.5, or 250 mg, and wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 21-67, 210-256 or 304-1099.

[0008] In some aspects, provided herein is a use of an antisense oligomer (ASO) for the manufacture of a medicament for treating or preventing a disease or condition characterized by a reduced expression or function of Nav1.1 protein in a human subject in need thereof, wherein the medicament is prepared to be administered at a first dose of about 0.1, 0.5, 1, 2.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, 57.5, 60, 62.5, 65, 67.5, 70, 72.5, 75, 77.5, 80, 82.5, 85, 87.5, 90, 92.5, 95, 97.5, 100, 102.5, 105, 107.5, 110, 112.5, 115, 117.5, 120, 122.5, 125, 127.5, 130, 132.5, 135, 137.5, 140, 142.5, 145, 147.5, 150, 152.5, 155, 157.5, 160, 162.5, 165, 167.5, 170, 172.5, 175, 177.5, 180, 182.5, 185, 187.5, 190, 192.5, 195, 197.5, 200, 202.5, 205, 207.5, 210, 212.5, 215, 217.5, 220, 222.5, 225,

mRNA) that is degraded in the cytoplasm, thus leading to no target protein production. **FIG. 1B** shows an example of the same cell divided into nuclear and cytoplasmic compartments. Treatment with a therapeutic agent, such as an antisense oligomer (ASO), promotes the exclusion of the nonsense-mediated mRNA decay-inducing exon from the pre-mRNA and results in an increase in processed mRNA, which is in turn translated into higher levels of target protein.

[0152] **FIG. 1C** is a schematic representation of therapeutic ASO-mediated exclusion of a nonsense-mediated mRNA decay-inducing exon from a pre-mRNA, which decreases non-productive processed mRNA (e.g., with an NMD exon) and increases productive mRNA (e.g., without an NMD exon) and increases expression of the full-length target protein from the productive mRNA.

[0153] **FIG. 1D** shows identification of an exemplary sequence in the *SCN1A* gene that encodes a nonsense-mediated mRNA decay (NMD)-inducing exon. The identification of the sequence in the *SCN1A* gene that encodes the NMD-inducing exon using comparative genomics is shown, visualized in the UCSC genome browser. The upper panel shows a graphic representation of the *SCN1A* gene to scale. The conservation level across 100 vertebrate species is shown as peaks. The highest peaks correspond to exons (black boxes), while no peaks are observed for the majority of the introns (lines with arrow heads). Peaks of conservation were identified in intron 20 (NM_006920), shown in the middle panel. Inspection of the conserved sequences identified an exon-like sequence of 64 bp (bottom panel, sequence highlighted in grey) flanked by 3' and 5' splice sites (underlined sequence). Inclusion of this exon leads to a frameshift and the introduction of a premature termination codon in exon 21 rendering the transcript a target of NMD. **FIG. 1D** discloses SEQ ID NO: 1100.

[0154] **FIG. 2** shows a study design timeline for monitoring wild type (WT) and Dravet Syndrome (DS) mice as well as a Kaplan-Meier curve showing DS and WT littermate mice monitored to 14 weeks for survival.

[0155] **FIG. 3** shows an experimental design for the EEG seizure monitoring study in DS mice and their WT littermates.

[0156] **FIGs. 4A-4E** show the results of monitoring seizure in mice administered with ASO-22 or PBS. **FIG. 4A** shows exemplary ECG recordings in DS mice. **FIG. 4B** shows the number of seizures occurring in various regions of the brain in the two mice groups. *Indicates $p < 0.05$. **FIG. 4C** summarizes total number of spontaneous seizures (generalized and focal) recorded between P22 and P46 in DS mice dosed with PBS (n=21) or ASO-22 (n=21). *Indicates $p < 0.05$. **FIG. 4D** shows the number of mice that had a number of seizures in each group. **FIG. 4E** shows effect of ASO-22 on the latency to the first recorded seizure between P22 and P46 in DS mice dosed with PBS (n=21) or ASO-22 (n=21).

[0157] **FIGs. 5A-5G** show that a single ICV injection of 20 μ g ASO-22 at P2 results in reduced SUDEP incidence and increased Nav1.1 protein expression in DS mice. **FIG. 5A** is a schematic for the experimental design. **FIGs. 5B, 5C, 5D, 5E, 5F, and 5G** illustrate the ASO-22 exposure, *Scn1a* expression, and Nav1.1 expression in brain tissues at 7 weeks or 14 weeks after a single ICV injection of ASO-22 (20 μ g) or PBS on P2, respectively.

[0158] **FIGs. 6A-6B** show the percent survival of DS and WT mice after a single ICV injection of ASO-22 (60 μ g) or PBS on P14.

CLAIMS

WHAT IS CLAIMED IS:

1. Use of an antisense oligomer (ASO) for the manufacture of a medicament for treating or reducing the likelihood of developing a disease or condition characterized by a reduced expression or function of Nav1.1 protein in a human subject in need thereof, wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 21-67, 210-256 or 304-1099, and wherein the medicament is to be administered at a dose of from about 0.5 milligrams to about 500 milligrams of the ASO.
2. The use according to claim 1, wherein multiple doses of the ASO are to be administered to the human subject.
3. The use according to claim 1, wherein the human subject is at most 18 years old.
4. The use according to claim 1, wherein the disease or condition is Dravet Syndrome.
5. The use according to claim 1, wherein the human subject is characterized by having:
 - (i) seizure onset prior to 12 months of age with recurrent focal motor or hemiclonic or generalized tonic-clonic seizures, which are often prolonged and triggered by hyperthermia;
 - (ii) no past history of causal magnetic resonance imaging lesion;
 - (iii) no other known etiology of any diseases or conditions except Dravet Syndrome;
 - (iv) normal development at seizure onset;
 - (v) a pathogenic variant, or variant of uncertain significance in an SCN1A gene;
 - (vi) at least 2 prior treatments for epilepsy that either had lack of adequate seizure control;
 - (vii) 4 or more convulsive seizures during the 28 days prior to administering, wherein the convulsive seizures is any one selected from Hemiclonic, Focal With Motor Signs, Focal To Bilateral Tonic Clonic Convulsion, Generalized Tonic Clonic Convulsion, Tonic, Tonic or Atonic (Drop Attacks), and Clonic;
 - (viii) a current intervention for epilepsy or medication with at least one antiepileptic drug at a dose which has been stable for at least 4 weeks, wherein the interventions for epilepsy is a ketogenic diet, a vagal nerve stimulator, or a cannabinoid or marijuana-derived product; or
 - (ix) any combination of (i) – (viii).

6. The use according to claim 1, wherein the human subject is characterized by not having one or more of the following:
- (a) one of the following mutations in the SCN1A gene: Thr226Met, Leu263Val, Val422Leu, Thr1174Ser, Trp1204Arg, Pro1345Ser, Gln1489Lys, Phe1499Leu, Arg1575Cys, Val1611Phe, Leu1624Pro, Arg1648Cys, Leu1649Gln, Leu1670Trp, Gly1674Arg, and Asp1866Tyr;
 - (b) a known pathogenic mutation in another gene that causes epilepsy, wherein the pathogenic mutation is homozygous in cases of known recessive disease;
 - (c) currently treated with a sodium channel blocker as maintenance treatment and an anticoagulant, wherein the sodium channel blocker is phenytoin, carbamazepine, oxcarbazepine, lamotrigine, lacosamide, or rufinamide, and wherein the anticoagulant is not an aspirin;
 - (d) clinically, significantly unstable medical conditions other than epilepsy;
 - (e) clinically, relevant symptoms or a clinically significant illness in the 4 weeks prior to administration, other than epilepsy;
 - (f) a history of brain or spinal cord disease other than epilepsy, Dravet Syndrome or a history of bacterial meningitis or brain malformation;
 - (g) a spinal deformity or other condition that alters the free flow of cerebrospinal fluid (CSF) or having an implanted CSF drainage shunt;
 - (h) clinically significant abnormal laboratory values prior to administration;
 - (i) aspartate aminotransferase or alanine aminotransferase >2.5-fold upper limit of normal, serum creatinine greater than an upper limit of normal or platelet count less than a lower limit of normal;
 - (j) clinically relevant abnormalities in the 12-lead electrocardiogram (ECG) measured at prior to administration;
 - (k) a psychiatric or behavioral disorder;
 - (l) currently or in the past 4 weeks, medication of an anticoagulant, wherein the anticoagulant is not aspirin; or
 - (m) any combination of (a) – (l).

7. The use according to claim 1, wherein the pharmaceutical composition is to be administered into the intrathecal space of the human subject.
8. The use according to claim 1, wherein the pharmaceutical composition is to be administered into the cerebrospinal fluid of the human subject.
9. The use according to claim 8, wherein the pharmaceutical composition is to be administered into the cerebrospinal fluid in the brain of the human subject.
10. The use according to claim 1, wherein the pharmaceutical composition is to be administered as a bolus injection.
11. The use according to claim 1, wherein the pharmaceutical composition is to be administered by infusion with a delivery pump.
12. The use according to claim 1, wherein the disease or condition is Alzheimer's Disease.
13. The use according to claim 1, wherein the administration reduces or ameliorates seizure frequency, seizure intensity, or seizure duration.
14. The use according to claim 1, wherein the administration treats seizure in the human subject suffering from Alzheimer's Disease.
15. The use according to claim 1, wherein the ASO comprises at least one modified sugar moiety.
16. The use according to claim 1, wherein the ASO comprises a 2'-O-methoxyethyl moiety.
17. The use according to claims 1, wherein the ASO comprises a thymidine comprising a 2'-O-methoxyethyl moiety.
18. The use according to claim 1, wherein each nucleobase of the ASO comprises a 2'-O-methoxyethyl moiety.
19. The use according to claim 1, wherein the ASO consists of from 16 to 20 nucleobases.
20. The use according to claim 1, wherein each cytosine of the ASO is a 5'-methylcytosine (5'-MeC).
21. The use according to claim 1, wherein each internucleoside linkage of the ASO is a phosphorothioate linkage.
22. The use according to claim 1, wherein a subsequent dose of from 0.5 milligrams to 500 milligrams of the ASO is to be administered to the human subject.
23. The use according to claim 22, wherein the subsequent dose is to be administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months after administration of the previous dose.

24. The use according to claim 1, wherein the pharmaceutical composition comprises from 0.1 mL to 50 mL of a diluent.
25. The use according to claim 1, wherein the pharmaceutical composition is to be administered to the human subject as a bolus injection over 1 to 60 minutes, 1 to 50 minutes, 1 to 40 minutes, 1 to 30 minutes, 1 to 20 minutes, 1 to 10 minutes, 1 to 5 minutes, or 1 to 3 minutes.
26. The use according to claim 1, wherein the pharmaceutical composition is to be administered to the human subject as a bolus injection using a spinal anesthesia needle.
27. The use according to claim 1, wherein the ASO is diluted in an artificial cerebral spinal fluid (aCSF) solution.
28. The use according to claim 1, wherein the ASO is diluted in a phosphate-buffered solution with a pH of from 6.6 – 7.6.
29. The use according to claim 1, wherein the ASO is diluted in a buffer comprising 25-250 mM NaCl, 0.1-20 mM KCl, 0.1-50 mM CaCl₂, and 0.1-50 mM MgCl₂.
30. The use according to claim 1, wherein the pharmaceutical formulation does not comprise a preservative.
31. The use according to claim 1, wherein the ASO is present in the pharmaceutical composition at a concentration of from 0.1 mg/mL to 250 mg/mL.
32. A pharmaceutical formulation comprising:
 - (a) an antisense oligomer (ASO), wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 21-67, 210-256 or 304-1099; and
 - (b) a pharmaceutically acceptable diluent;wherein the ASO is present in the pharmaceutical formulation at a concentration of from 0.1 mg/mL to 200 mg/mL.
33. The pharmaceutical formulation of claim 32, wherein the pharmaceutically acceptable diluent comprises an artificial cerebral spinal fluid (aCSF) solution.
34. The pharmaceutical formulation of claim 32, wherein the solution comprises a cerebral spinal fluid (CSF) sample from the subject.
35. The pharmaceutical formulation of claim 32, wherein the ASO is solubilized or diluted in an isotonic solution.

36. The pharmaceutical formulation of claim 32, wherein the ASO is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution.
37. The pharmaceutical formulation of claim 32, wherein the ASO is solubilized or diluted in a buffer comprising 25-250 mM NaCl, 0.1-20 mM KCl, 0.1-50 mM Na₂HPO₄, 0.1-50 mM NaH₂PO₄, 0.1-50 mM CaCl₂, and 0.1-50 mM MgCl₂.
38. The pharmaceutical formulation of claim 32, wherein the ASO is solubilized or diluted in a buffer comprising carbohydrates.
39. The pharmaceutical formulation of claim 32, wherein the ASO is solubilized or diluted in a buffer comprising an antioxidant.
40. The pharmaceutical formulation of claim 32, wherein the pharmaceutical formulation does not comprise a preservative.
41. The pharmaceutical formulation of claim 32, wherein the pharmaceutical formulation is suitable for an intracerebroventricular or intrathecal injection.
42. The pharmaceutical formulation of claim 32, wherein the pharmaceutical formulation is packaged in a single use vial.
43. A kit comprising:
 - (i) an concentrate comprising an antisense oligomer (ASO), wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 21-67, 210-256 or 304-1099; and
 - (ii) a diluent, wherein the concentrate is miscible with the diluent; and
 - (iii) instructions for diluting or solubilizing the ASO in the diluent.