

ANTISENSE OLIGOMERS FOR TREATMENT OF CONDITIONS AND DISEASES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/811,511, filed on February 27, 2019 which is incorporated herein by reference in its entirety.

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, 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

[0002a] In one aspect, the present disclosure refers to ~~a~~an in-vitro method of modulating expression of Nav1.1 protein in a cell having a pre-mRNA that contains a non-sense mediated mRNA decay-inducing exon (NMD exon) and that encodes Nav1.1 protein, the method comprising contacting a therapeutic agent or a viral vector encoding the therapeutic agent to the cell, whereby the therapeutic agent ~~modulates-promotes~~ exclusion of the NMD exon from the pre-mRNA encoding Nav1.1 protein, thereby ~~modulating-increasing~~ a level of processed mRNA encoding Nav1.1 protein, and ~~modulating~~ increasing expression of Nav1.1 protein in the cell, wherein the therapeutic agent binds to a targeted region of the pre-mRNA encoding Nav1.1, and wherein the targeted region is: from ~~about~~-1000 nucleotides upstream from the 5' end of the NMD exon to ~~about~~-100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~-100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~-1000 nucleotides downstream of the 3' end of the NMD exon, wherein the therapeutic agent comprises an antisense oligomer (ASO), and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.

[0002b] In another aspect, the present disclosure refers to a therapeutic agent or viral vector encoding a therapeutic agent for use in therapy, wherein the therapeutic agent ~~modulates-promotes~~ splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from a -pre-mRNA in a cell of the subject that contains the NMD exon and that encodes Nav1.1 protein, thereby ~~modulating-increasing~~ the level of processed mRNA encoding the Nav1.1 protein, and ~~modulating-increasing~~ expression of

Nav1.1 protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the pre-mRNA encoding the Nav1.1 protein, and wherein the targeted portion is: from ~~about~~-1000 nucleotides upstream from the

5' end of NMD exon to ~~about~~-100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~-100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~-1000 nucleotides downstream of the 3' end of the NMD exon, wherein the therapeutic agent comprises an antisense oligomer (ASO), and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.

[0002c] In another aspect, the present disclosure refers to a use of a therapeutic agent or viral vector encoding a therapeutic agent in the manufacture of a medicament for the treatment of a disease or condition associated with haploinsufficiency of the *SCN1A* gene, wherein the therapeutic agent ~~modulates-promotes~~ splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from a pre-mRNA in a cell of the subject that contains the NMD exon and that encodes Nav1.1 protein, thereby ~~modulating-increasing~~ the level of processed mRNA encoding the Nav1.1 protein, and ~~modulating-increasing~~ expression of Nav1.1 protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the pre-mRNA encoding the Nav1.1 protein, and wherein the targeted portion is: from ~~about~~-1000 nucleotides upstream from the 5' end of NMD exon to ~~about~~-100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~-100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~-1000 nucleotides downstream of the 3' end of the NMD exon, wherein the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); Febrile seizures, familial, 3A; epilepsy, generalized, with febrile seizures plus (GEFS+); epilepsy, generalized, with febrile seizures plus, type 2; encephalopathy; epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy, wherein the therapeutic agent comprises an antisense oligomer (ASO) and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.

[0002d] In another aspect, the present disclosure refers to a therapeutic agent or a viral vector encoding the therapeutic agent; wherein the therapeutic agent binds to a targeted portion of a pre-mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon) and that encodes Nav1.1 protein, whereby the therapeutic agent ~~modulates-promotes~~ exclusion of the NMD exon from the pre-mRNA encoding Nav1.1, and wherein the targeted portion is: from ~~about~~-1000 nucleotides upstream

from the 5' end of an NMD exon to ~~about~~ 100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~ 100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~ 1000 nucleotides downstream of the 3' end of the NMD exon, wherein the therapeutic agent comprises an antisense oligomer (ASO), and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.

[0003] Disclosed herein, in certain examples, is a method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE. In some examples, the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion. In some examples, the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NIE. In some examples, the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides,

CLAIMS

What is claimed is:

1. ~~A~~ An *in-vitro* method of modulating expression of Nav1.1 protein in a cell having a pre-mRNA that contains a non-sense mediated mRNA decay-inducing exon (NMD exon) and that encodes Nav1.1 protein, the method comprising contacting a therapeutic agent or a viral vector encoding the therapeutic agent to the cell, whereby the therapeutic agent ~~modulates~~ promotes exclusion of the NMD exon from the pre-mRNA encoding Nav1.1 protein, thereby ~~modulating~~ increasing a level of processed mRNA encoding Nav1.1 protein, and ~~modulating~~ increasing expression of Nav1.1 protein in the cell, wherein the therapeutic agent binds to a targeted region of the pre-mRNA encoding Nav1.1, and wherein the targeted region is: from ~~about~~ 1000 nucleotides upstream from the 5' end of the NMD exon to ~~about~~ 100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~ 100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~ 1000 nucleotides downstream of the 3' end of the NMD exon, wherein the therapeutic agent comprises an antisense oligomer (ASO), and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.
2. ~~The method of claim 1, wherein the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted region.~~
3. ~~The method of claim 1, wherein the targeted region is at most 500 nucleotides, upstream of 5' end of the NMD exon.~~
4. ~~The method of claim 1, wherein the targeted region is at least 500 nucleotides upstream of 5' end of the NMD exon.~~
5. ~~The method of claim 1, wherein the targeted region is at most 500 nucleotides downstream of 3' end of the NMD exon.~~
6. ~~The method of claim 1, wherein the targeted region is at least 500 nucleotides downstream of 3' end of the NMD exon.~~
7. ~~The method of any one of claims 1-6, wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.~~
8. ~~The method of any one of claims 1-7, wherein the targeted region is within an intronic sequence flanking the NMD exon.~~

- ~~9. The method of any one of claims 1-8, wherein the targeted region is comprised within sequence with at least 95% sequence identity to SEQ ID NO: 6.~~
- ~~10. The method of any one of claims 1-9, wherein the therapeutic agent is an antisense oligomer (ASO).~~
- ~~11. The method of claim 10, wherein the ASO comprises a sequence that is at least about 95% identical to any one of SEQ ID NOs: 12-731.~~
- ~~12. The method of any one of claims 1-11, wherein the therapeutic agent promotes exclusion of the NMD exon from the pre-mRNA.~~
- ~~13. The method of claim 12, wherein the therapeutic agent increases a level of the processed mRNA encoding Nav1.1 protein in the cell.~~
- ~~14. The method of claim 13, wherein the therapeutic agent increases a level of Nav1.1 protein in the cell.~~
- ~~15. The method of claim 14, wherein an amount of the Nav1.1 protein in the cell contacted with the therapeutic agent is increased at least 1.1-fold compared to a total amount of Nav1.1 protein in a control cell.~~
- ~~16. The method of any one of claims 1-15, wherein the ASO consists of a sequence selected from the group consisting of SEQ ID NOs: 72, 73, 76, 181, 220, 432, 433, 436, 541 and 580.~~
- ~~17. The method of any one of claims 1-16, wherein the method comprises contacting a vector encoding the therapeutic agent to the cell, wherein the vector is a viral vector.~~
- ~~182.~~ A therapeutic agent or viral vector encoding a therapeutic agent for use in therapy, wherein the therapeutic agent ~~modulates~~ promotes splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from a pre-mRNA in a cell of the subject that contains the NMD exon and that encodes Nav1.1 protein, thereby ~~modulating-increasing~~ the level of processed mRNA encoding the Nav1.1 protein, and ~~modulating-increasing~~ expression of Nav1.1 protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the pre-mRNA encoding the Nav1.1 protein, and wherein the targeted portion is: from ~~about~~ 1000 nucleotides upstream from the 5' end of NMD exon to ~~about~~ 100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~ 100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~ 1000 nucleotides downstream of the 3' end of the NMD exon, wherein the therapeutic agent comprises an antisense

- oligomer (ASO), and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.
3. The therapeutic agent of claim 2, wherein the ASO comprises a sequence that is at least 95% identical to any one of SEQ ID NOs: 12-731.
4. The therapeutic agent of claim 2, wherein the ASO consists of a sequence selected from the group consisting of SEQ ID NOs: 72, 73, 76, 181, 220, 432, 433, 436, 541 and 580.
495. Use of a therapeutic agent or viral vector encoding a therapeutic agent in the manufacture of a medicament for the treatment of a disease or condition associated with haploinsufficiency of the *SCN1A* gene, wherein the therapeutic agent ~~modulates-promotes~~ splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from a pre-mRNA in a cell of the subject that contains the NMD exon and that encodes Nav1.1 protein, thereby ~~modulating-increasing~~ the level of processed mRNA encoding the Nav1.1 protein, and ~~modulating-increasing~~ expression of Nav1.1 protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the pre-mRNA encoding the Nav1.1 protein, and wherein the targeted portion is: from ~~about~~-1000 nucleotides upstream from the 5' end of NMD exon to ~~about~~-100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~-100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~-1000 nucleotides downstream of the 3' end of the NMD exon, wherein the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); Febrile seizures, familial, 3A; epilepsy, generalized, with febrile seizures plus (GEFS+); epilepsy, generalized, with febrile seizures plus, type 2; encephalopathy; epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy, wherein the therapeutic agent comprises an antisense oligomer (ASO) and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.
6. The use according to claim 5, wherein the haploinsufficiency is associated with a loss-of-function mutation in Nav1.1 protein.

7. The use according to claim 5, wherein the ASO interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted region.
8. The use according to claim 5, wherein the targeted region is at most 500 nucleotides upstream of 5' end of the NMD exon.
9. The use according to claim 5, wherein the targeted region is at least 500 nucleotides upstream of 5' end of the NMD exon.
10. The use according to claim 5, wherein the targeted region is at most 500 nucleotides downstream of 3' end of the NMD exon.
11. The use according to claim 5, wherein the targeted region is at least 500 nucleotides downstream of 3' end of the NMD exon.
12. The use according to any one of claims 5-11, wherein the targeted region is within sequence with at least 95% sequence identity to SEQ ID NO: 6.
13. The use according to claim 5, wherein an amount of the Nav1.1 protein in the cell contacted with the ASO is increased at least 1.1-fold compared to a total amount of Nav1.1 protein in a control cell.
14. The use according to any one of claims 5-13, wherein the ASO comprises a sequence that is at least 95% identical to any one of SEQ ID NOs: 12-731.
15. The use according to any one of claims 5-14, wherein the ASO consists of a sequence selected from the group consisting of SEQ ID NOs: 72, 73, 76, 181, 220, 432, 433, 436, 541 and 580.
- ~~20. The use according to claim 19, wherein the disease or condition is associated with a loss of function mutation in Nav1.1 protein.~~
- ~~21. The use according to claim 19 or 20, wherein the subject has a first allele encoding a functional Nav1.1 protein, and a second allele from which Nav1.1 protein is not produced or produced at a reduced level, or a second allele encoding a nonfunctional Nav1.1 protein or a partially functional Nav1.1 protein.~~
- ~~22. The use according to any one of claims 19-21, wherein the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI) borderland (SMEB); Febrile seizure (FS); Febrile seizures, familial, 3A; epilepsy, generalized, with febrile seizures plus (GEFS+); epilepsy, generalized, with febrile seizures plus, type 2; encephalopathy; epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy;~~

~~myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy.~~

~~2316.~~ A therapeutic agent or a viral vector encoding the therapeutic agent; wherein the therapeutic agent binds to a targeted portion of a pre-mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon) and that encodes Nav1.1 protein, whereby the therapeutic agent ~~modulates-promotes~~ exclusion of the NMD exon from the pre-mRNA encoding Nav1.1, and wherein the targeted portion is: from ~~about~~ 1000 nucleotides upstream from the 5' end of an NMD exon to ~~about~~ 100 nucleotides upstream from the 5' end of the NMD exon; or from ~~about~~ 100 nucleotides downstream of the 3' end of the NMD exon to ~~about~~ 1000 nucleotides downstream of the 3' end of the NMD exon, wherein the therapeutic agent comprises an antisense oligomer (ASO), and wherein the NMD exon comprises a sequence with at least 95% sequence identity to SEQ ID NO: 7.

17. The therapeutic agent of claim 16, wherein the ASO comprises a sequence that is at least 95% identical to any one of SEQ ID NOs: 12-731.

18. The therapeutic agent of claim 16, wherein the ASO consists of a sequence selected from the group consisting of SEQ ID NOs: 72, 73, 76, 181, 220, 432, 433, 436, 541 and 580.