

OLIGONUCLEOTIDES FOR MODULATING KCNT1 EXPRESSION

BACKGROUND OF THE INVENTION

KCNT1 gene encodes an intracellular sodium-activated potassium channel (potassium sodium-activated channel subfamily T member 1- UNIPROT ID Q5JUK3-
5 3) that is expressed in the central nervous system. Also known as Slack, *KCNT1* is a member of the Slo-type family of potassium channel genes and can co-assemble with other Slo channel subunits. These channels can mediate a sodium-sensitive potassium current, which is triggered by an influx of sodium channels ions through sodium channels or neurotransmitter receptors. Delayed outward current may be
10 involved in regulating neuronal excitability.

Pathogenic variants in the *KCNT1* gene encoding the potassium sodium-activated channel subfamily T member 1 are associated with a spectrum of epilepsies and neurodevelopment disorders (Barcia et al., *Neurol Genet.* (2019) 5(6):e363). They cause developmental and epileptic encephalopathies (DEEs)
15 including epilepsy of infancy with migrating focal seizures (EIMFS) and early onset epileptic encephalopathy (EOEE). DEEs associated with *KCNT1* pathogenic variants are characterized by normal prenatal development and birth. EIMFS, the most frequent and severe DEE phenotype, usually presents in early infancy and is defined by an extremely high seizure burden. Similar to EIMFS, patients with EOEE usually
20 present with symptoms before one year of age and have a high seizure burden and experience seizures almost daily; these patients also have severe developmental delays and early mortality (Bonardi et al., *Brain* (2021) 144(12):3635-3650). A recent review of the literature reports 189 individuals affected by *KCNT1* associated DEEs (Bonardi, *supra*), although the actual number of patients may be higher, with The
25 *KCNT1* Foundation reporting 3,000 cases worldwide. This incidence rate would characterize DEE as an ultra-rare disease.

Typically, DEE disease progression occurs in three phases: a sporadic seizure phase, a stormy phase, and a chronic phase. In the stormy phase, patients can experience hundreds of seizures a day, which usually arises between three and
30 six months of life. This phase is characterized by frequent and/or prolonged seizures or status epilepticus, leading to severe developmental delay and an increased risk of death. The chronic phase typically occurs around 1-2 years of age and patients experience fewer seizures. However, a higher level of clinical heterogeneity is observed, and some patients enter the stormy phase without the sporadic phase and

the time course of the different phases can be highly variable among the affected children (Kuchenbuch et al., *Brain* (2019) 142(10):2996-3008). Death may occur at any stage in the disease course. Death may have different causes including heart failure and other comorbidities, but sudden unexpected death in epilepsy has been reported as being the most prominent in EIMFS.

The seizures are primarily focal motor, with variable secondary generalization, including tonic, clonic, tonic clonic, myoclonic and epileptic spasms. In the chronic phase, the seizures are mainly tonic with dysautonomic manifestations. For example, perioral cyanosis and apnea are common. The characteristic features on electroencephalograms (EEGs) are focal ictal discharges that migrate across neighbouring cortical regions and may arise independently at multiple loci. Other neurological features in these patients include hypotonia, microcephaly, and severe developmental disabilities. Variable delayed myelination, hippocampal volume loss, and cerebellar atrophy have been noted on brain magnetic resonance imaging (MRI).

Seizure control has been attempted with multiple interventions, including benzodiazepines, vigabatrin, stiripentol, phenobarbital, topiramate, quinidine, and a ketogenic diet. Their lack of efficacy has been widely reported (Landmark et al., *Epilepsia* (2021) 62(4):857-873). Seizures can become rapidly resistant to the medications. Quinidine, an anti-arrhythmic drug, was explored as an anti-seizure treatment in DEEs, but in some patients it worsened the disease or even caused serious adverse events (e.g., QT interval prolongation) (Liu et al., *Neurol Sci.* (2023) 44(4):1201-1206). Valeriasen, an antisense oligonucleotide that was designed to degrade *KCNT1* mRNA, was administered in two pediatric carriers of *KCNT1* mutants who responded with a decrease of seizure number. However, treatment had to be paused due to the emergence of severe adverse events including hydrocephalus.

DEE associated with *KCNT1* pathogenic variants is a devastating pediatric neurodevelopmental disorder with prognosis varying from severe encephalopathy to early death. This dismal prognosis and a lack of effective therapies highlight the urgent medical need for this disease.

SUMMARY OF THE INVENTION

The present disclosure provides antisense oligonucleotides (ASOs) that reduce the abundance or activity of RNA transcribed from the *KCNT1* gene. By reducing levels of *KCNT1* RNA, the compounds of the present disclosure decrease the abundance of KCNT1 protein in the cell, thereby reducing the activity of the channel and hyperexcitability associated to the mutated channels. The compounds may alleviate the symptoms and/or delay disease progression.

In some aspects, the present disclosure provides an antisense oligonucleotide reducing *KCNT1* expression, wherein the antisense oligonucleotide has a nucleobase sequence that comprises at least 12 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 4-12.

In particular embodiments, the antisense oligonucleotide described herein has a nucleobase sequence that comprises at least 15 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 4-12.

In some embodiments, the nucleobase sequence of the antisense oligonucleotide may be selected from SEQ ID NOs: 4-12. In further embodiments, the antisense oligonucleotide has a nucleobase sequence of any one of SEQ ID NOs: 7, 8, and 9.

In some embodiments, the antisense oligonucleotide described herein has 18 to 20 linked nucleosides.

In some embodiments, an oligonucleotide described herein may comprise a modified internucleoside linkage, e.g., a phosphodiester internucleoside linkage. In some embodiments, an oligonucleotide described herein may comprise phosphodiester internucleoside linkage(s) and/or phosphorothioate internucleoside linkage(s). In certain embodiments, the oligonucleotide may comprise at least 1, 2, 3, 4, 5 or 6 phosphodiester internucleoside linkages. In certain embodiments, at least 1, 2, 3, 4, 5, or more, or all internucleoside linkages in the oligonucleotide are phosphorothioate internucleoside linkages.

In certain embodiments, the phosphorothioate internucleoside linkages are at one or more, or all, of positions 1-2 (i.e., between nucleosides 1 and 2), 5-16 (i.e., between adjacent nucleosides starting at nucleoside 5 and ending at nucleoside 16; that is, between nucleosides 5 and 6, nucleosides 6 and 7, nucleosides 7 and 8, nucleosides 8 and 9, nucleosides 9 and 10, nucleosides 10 and 11, nucleosides 11 and 12, nucleosides 12 and 13, nucleosides 13 and 14, nucleosides 14 and 15, and

nucleosides 15 and 16), and 19-20 (i.e., between nucleosides 19 and 20) in the antisense oligonucleotide of the present disclosure.

In some embodiments, the antisense oligonucleotide described herein has at least one nucleoside comprising a modified sugar moiety (e.g., a modified ribose or modified deoxyribose moiety). In further embodiments, the modified sugar moiety comprises a 2'-O-methoxyethyl group (e.g., 2'-O-methoxyethyl ribose).

In some embodiments, an oligonucleotide described herein comprises the following formula:

i) Aes Teo mCeo mCeo mCes Ads Gds Gds Tds Tds Tds Ads mCds mCds mCds Geo Ae Teo Tes mCe (SEQ ID NO: 7);

ii) Tes mCeo mCeo mCeo Aes Gds Gds Tds Tds Tds Ads mCds mCds mCds Gds Ae Teo Teo mCes Ae (SEQ ID NO: 8);

iii) Tes Ae Teo mCeo mCes mCds Ads Gds Gds Tds Tds Tds Ads mCds mCds mCeo Geo Ae Teo Tes Te (SEQ ID NO:9);

iv) Aes Teo mCeo mCeo mCes Ads Gds Gds Tds Tds Tds Ads Cds Cds Cds Geo Ae Teo Tes mCe (SEQ ID NO:10);

v) Tes mCeo mCeo mCeo Aes Gds Gds Tds Tds Tds Ads Cds Cds Cds Gds Ae Teo Teo mCes Ae (SEQ ID NO: 11);

vi) Tes Ae Teo mCeo mCes Cds Ads Gds Gds Tds Tds Tds Ads Cds Cds mCeo Geo Ae Teo Tes Te (SEQ ID NO:12)

wherein

A = an adenine

C = a cytosine

mC = a 5-methylcytosine

G = a guanine

T = a thymine

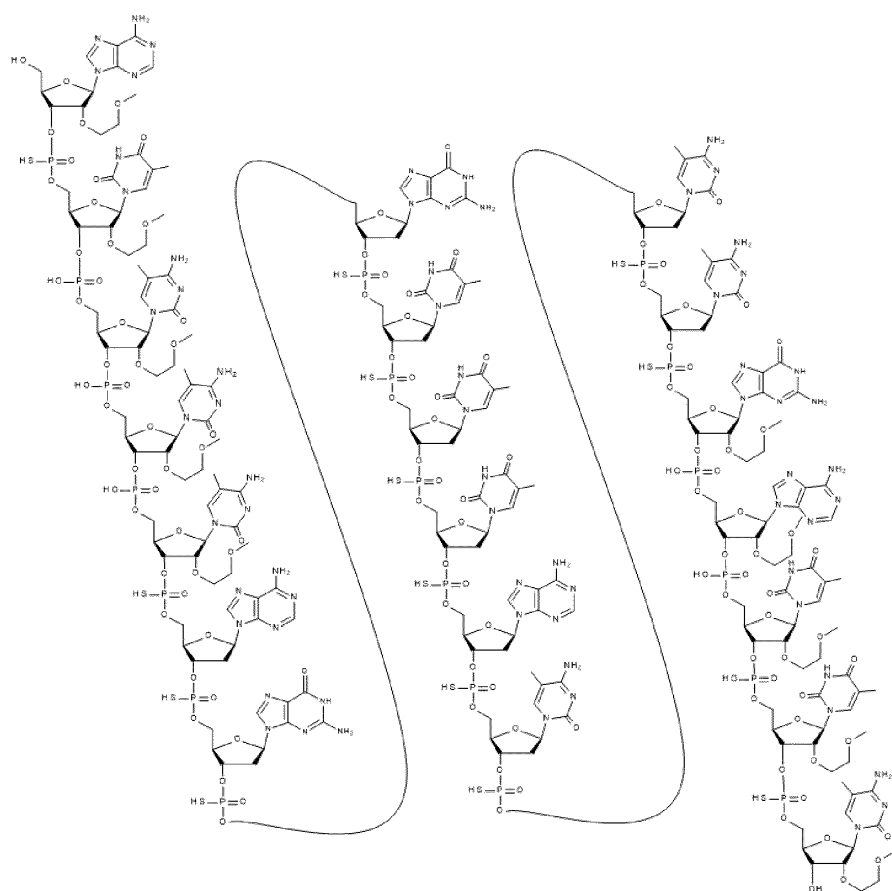
e = a 2'-O-methoxyethylribose modified sugar

d = a 2'-deoxyribose

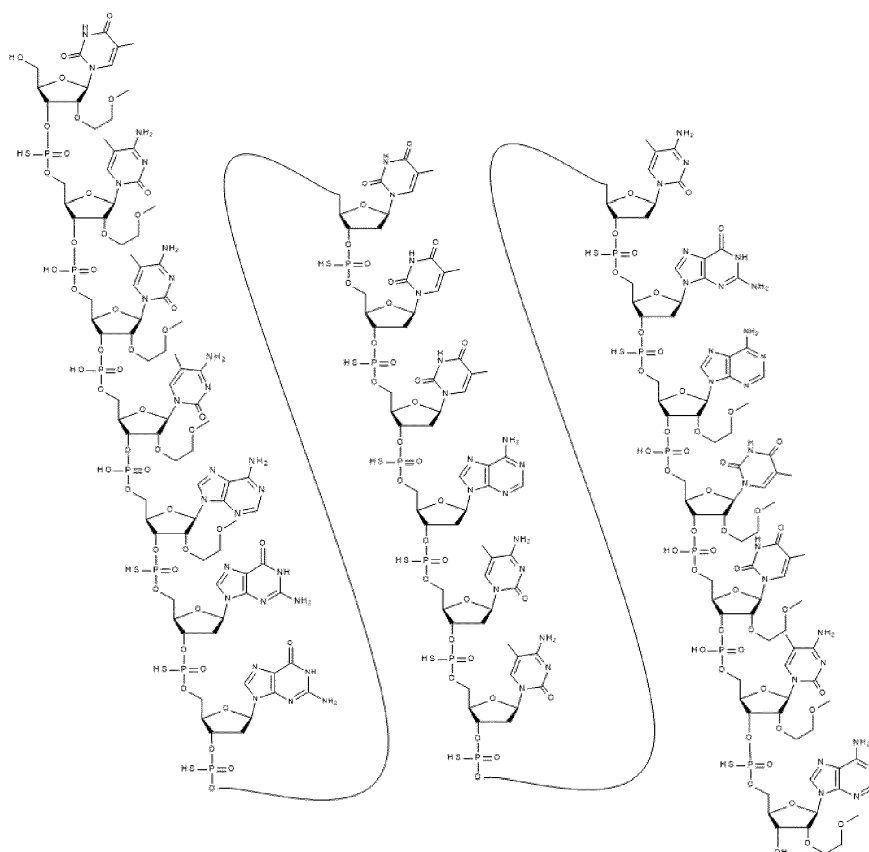
s = a phosphorothioate internucleoside linkage

o = a phosphodiester internucleoside linkage.

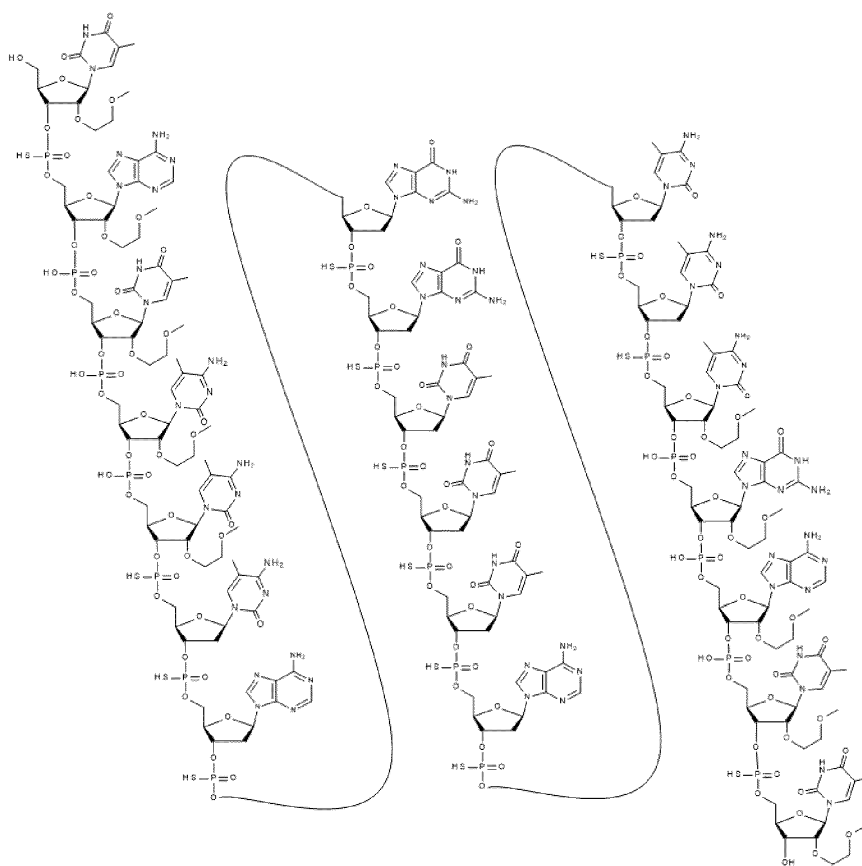
In some embodiments, the present disclosure provides an oligonucleotide comprising the structural formula:



In some embodiments, the present disclosure provides an oligonucleotide comprising the structural formula:



In some embodiments, the present disclosure provides an oligonucleotide comprising the structural formula:



The present disclosure also provides an oligonucleotide conjugate comprising an antisense oligonucleotide described herein wherein at least one conjugate moiety
5 is covalently attached to said oligonucleotide.

In some aspects, the present disclosure provides a pharmaceutical composition comprising an oligonucleotide described herein or a conjugate as described herein and a pharmaceutically acceptable excipient.

Also provided is a method of reducing KCNT1 expression in a mammalian
10 cell, comprising contacting the cell with an antisense oligonucleotide, a conjugate or a pharmaceutical composition described herein, thereby reducing KCNT1 expression in the cell. In some embodiments, the cell is a central nervous system cell, such as a cell in the human brain.

In some embodiments, the present disclosure provides a method for treating a
15 DEE in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antisense oligonucleotide, a conjugate or a

pharmaceutical composition described herein. The oligonucleotide, conjugate, or pharmaceutical composition may be, e.g., injected intrathecally or intracranially to the subject.

It is understood that any of the antisense oligonucleotides, conjugates, or pharmaceutical compositions described herein may be used in any methods described herein for the manufacture of a medicament for treating a DEE in a (human) subject in need thereof. In some embodiments, the oligonucleotide, the conjugate or the pharmaceutical composition described herein may be used in any treatment of DEE (e.g., EIMFS or EOEE) in a (human) subject in need thereof.

Other features, objectives, and advantages of the invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments and aspects of the invention, is given by way of illustration only, not limitation. Various changes and modification within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE FIGURES

FIG.1 is a pair of graphs showing dose-dependent percent reduction of human *KCNT1* RNA upon treatment with the indicated *KCNT1* ASOs.

FIG. 2 is pair of bar graphs comparing the mRNA reduction in wildtype (WT) and mutated P924L induced pluripotent stem (iPS) cell derived neurons by *KCNT1*_ASO_00849 and *KCNT1*_Valeriasen.

FIG. 3 is a table showing the tolerability scoring system for mice and rats utilized in the *in vivo* assays described herein.

FIG. 4 is a pair of bar graphs showing the efficacy and tolerability of the tested ASOs in mice. The left Y axis and solid bars depict the expression level of *KCNT1* mRNA expressed in mouse neurons *in vivo* two or six weeks after treatment with the ASOs relative to PBS-treated samples. The right Y axis and black circles depict the functional observational battery (FOB) absolute score observed in mice one hour after treatment with the ASOs.

FIG. 5 is panel of bar graphs showing the efficacy of *KCNT1*_ASO_0815, *KCNT1*_ASO_0849, and *KCNT1*_Valeriasen at doses of 1, 5, 10, 30 and 100 nmol. The left Y axis and solid bars depict the expression level of *KCNT1* mRNA

expressed in mouse neurons *in vivo* four weeks after treatment with the ASOs relative to PBS-treated samples.

FIG. 6 is a dot graph showing the concentration of KCNT1_ASO_00849 and KCNT1_Valeriasen quantified by HPLC fluorescence in cortical tissue homogenate
5 four weeks after a single injection of 5, 15, 30, 60, or 100 nmol of the ASOs.

FIG. 7 is a dot graph comparing inhibition of *KCNT1* mRNA expression in the cortex by KCNT1_ASO_00815 and KCNT1_ASO_00849 at the dose of 20 or 60 nmol as quantified by qRT-PCR.

FIG. 8 is a dose response graph that compares the KCNT1_Valeriasen and
10 KCNT1_ASO_00849 based on dose effect relationship on mRNA expression level in target tissue.

FIG. 9 is an anatomical set of MRI images of rat brain used to quantify the volume of lateral ventricles (LV). Volumes of LV were assessed by manual delineation on the MRI images. The rostral and caudal margins of the corpus
15 callosum served as anatomical landmarks to limit segmentation. Of the 70 sections covering the brain, approximately 35 to 40 sections were segmented to estimate LV volumes. the total LV volume is the sum of all these values. The top panel corresponds to T2-weighted brain images and the bottom panel corresponds to manually defined regions of interest delimitating LV which are colored in bright.

FIG.10 is a bar graph showing the total lateral ventricles (LV) volume following
20 administration of aCSF, KCNT1_Valeriasen or KCNT1_ASO_815. The left Y axis depicts the total volume of LV acquired at baseline, two weeks or four weeks after treatment with brain MRI.

25 DETAILED DESCRIPTION OF THE INVENTION

The present disclosure is based on the discovery that antisense oligonucleotides (ASOs) targeting RNAs transcribed from the *KCNT1* gene can effectively reduce the abundance of target *KCNT1* transcripts and/or translation of the KCNT1 polypeptide from the transcripts. The ASOs of the present disclosure
30 comprise sequences that are complementary to *KCNT1* transcripts and bind to defined nucleotide sequences within the transcripts.

By decreasing the level of *KCNT1* target transcripts in a cell, the ASO mediates a decrease in the expression of the KCNT1 protein in the cell, alleviating the severity or progression of epilepsies and neurodevelopment disorders. The

ASOs of the present disclosure are expected to be particularly useful in the treatment of DEE. The ASOs of the present disclosure are highly advantageous in that they target KCNT1 expression at the *KCNT1* transcript level and thus have the ability to decrease expression of the KCNT1 protein.

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I. The *KCNT1* Gene and KCNT1 Protein

The ASOs of the present disclosure bind to transcripts of the *KCNT1* gene, which encodes the KCNT1 protein. The *KCNT1* transcript named KCNT1-202 has the sequence set forth in SEQ ID NO: 1 (GENBANK Accession No: NM_020822.3, 10 ENST00000371757.7) and codes for the protein UNIPROT ID Q5JUK3-3 of 1235 amino acids. The transcript named KCNT1-212 (ENST00000628528.2, SEQ ID NO: 2) codes for the protein UNIPROT ID Q5JUK3-4 of 1211 amino acids. These two transcripts are mRNAs derived from the human *KCNT1* gene (ENSG00000107147, SEQ ID NO: 3). In some embodiments, an ASO described herein targets a transcript 15 of a mammalian *KCNT1* gene (e.g., a rodent or human *KCNT1* gene).

In some embodiments, an ASO of the present disclosure binds to an *KCNT1* gene sequence, or a transcript thereof. In some embodiments, an ASO of the present disclosure binds to an *KCNT1* transcript that encodes a KCNT1 protein, e.g., as found under UniProt Accession Number Q5JUK3-3 or Q5JUK3-4. In certain 20 embodiments, an ASO of the present disclosure comprises a sequence that may be at least 60, 70, 80, 85, 90, or 95%, or 100% complementary to a same-length sequence in the target *KCNT1* transcript.

In some embodiments, an ASO of the present disclosure can bind to a transcript of a wildtype or mutated *KCNT1* gene (e.g., a wildtype human, non-human 25 primate, or rodent gene). In some embodiments, an ASO of the present disclosure binds to a variant, such as a known variant, of the wildtype or mutated *KCNT1* gene. An ASO of the present disclosure has not been designed to target selectively a KCNT1 mutated transcript.

An ASO of the present disclosure complements with a perfect match to 30 wildtype *KCNT1* transcripts in other nonclinical model organisms including mouse, rat, and cynomolgus monkey.

The binding site of any ASOs herein was also examined for known variation in the human population. It contains seven polymorphisms that are not disease-associated (rs781622931, rs1588412829, rs746160320, rs770265176,

rs780485857, rs1834215203, and rs1337703203). Known disease-causing variants were not found within this region.

The present ASOs can reduce or inhibit expression of wildtype or variant *KNCT1* transcripts. In certain embodiments, an ASO described herein may reduce or inhibit expression of an *KNCT1* transcript encoding a KCNT1 protein such as KCNT1-202 or KCNT1-212.

The present ASOs comprise sequences that are complementary to a same-length sequence in a target transcript encoded by the *KNCT1* gene (wherein the genomic *KNCT1* sequence may comprise, e.g., SEQ ID NO: 3). In certain embodiments, an ASO described herein comprises a sequence that is complementary to a sequence in a hotspot region within the target *KNCT1* transcript. The term “hotspot region” refers to a region of the target nucleotide sequence wherein binding of a sequence within the region by a complementary ASO tends to result in a reduction in the abundance or translational activity of the target RNA transcript. A hotspot region may be entirely within an intron, entirely within an exon, or may span an intron/exon junction; or be located in whole or in part in the 5' or 3' untranslated region (UTR) of an RNA transcript. In certain embodiments, binding of a sequence in a hotspot region by an ASO described herein reduces *KNCT1* RNA levels by at least 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% in a cell (e.g., as determined in an *in vitro* assay such as the one described below in the section titled “Example of ASO Screenings in Neurons *in vitro*”).

In the present disclosure, an ASO compound is interchangeably referred to as KCNT1_ASO_[compound number] and as [compound number]. KCNT1_Valeriasen also is referred to as Valeriasen. For example, compound number KCNT1_ASO_00815 and compound number 00815 represent the same ASO compound.

II. Antisense Oligonucleotides

The term “antisense oligonucleotide” or “ASO” refers to an oligonucleotide capable of hybridizing to a sequence in a target transcript. It is understood by a person skilled in the art that the ASOs described herein do not occur in nature (i.e., they are “isolated” ASOs).

The term “transcript” refers to any RNA transcribed from a gene (e.g., an *KNCT1* gene). The gene may be wildtype or may be a mutated or variant (e.g.,

polymorphic) form. An RNA transcript may be a primary RNA transcript or precursor messenger RNA (pre-mRNA), or a messenger RNA (mRNA), and may include exons, introns, 5' UTRs and 3' UTRs. Unless otherwise indicated, the sequences of transcripts and ASOs provided herein denote the nucleotide sequence from 5' end
5 (left) to 3' end (right).

The term "oligonucleotide," as used in the present disclosure, refers to a compound comprising a strand of about 5 to 100 nucleosides, e.g., 5 to 50 nucleosides, e.g., 8 to 30 nucleosides, e.g., 20 nucleosides, connected via internucleoside linkages. Each nucleoside and internucleoside linkage of an
10 oligonucleotide of the present disclosure may be modified or unmodified from naturally occurring nucleotides and linkages. A modified oligonucleotide may comprise one or more modified sugar (e.g., ribose or deoxyribose) moieties, one or more modified nucleobases, and/or one or more modified internucleoside linkages.

An ASO described herein may comprise a sequence that is substantially or
15 fully complementary to a same-length sequence in the target transcript. Full complementarity occurs when a first strand of contiguous nucleotides (modified or unmodified) and a second strand of contiguous nucleotides (modified or unmodified) are completely complementary to each other over the entire length of the shorter strand (or both strands, if they are of the same length). The two strands are
20 considered substantially complementary to each other when they base-pair with each other over 80% or more (e.g., 90% or more) over the length of the shorter strand (or both strands, if they are of the same length), with no more than 20% (e.g., no more than 10%) of mismatching base-pairs (e.g., for a duplex of 20 nucleotides, no more than 4 or no more than 2 mismatched base-pairs). In some embodiments, a
25 sequence in an ASO of the present disclosure is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a target RNA transcript. In some embodiments, the present ASO comprises no more than 1, 2, 3 or 4 mismatches to its target sequence.

The term "identical" or "identity" in the context of comparing two nucleotide
30 sequences refers to identical nucleobases. The term "percent identity" in this context refers to the percentage of nucleobases that are the same when the two comparing sequences are aligned (introducing gaps, if necessary) for maximum correspondence, over the length of the shorter comparing sequence (or both sequences, if the comparing sequences are of the same length).

In certain embodiments, reduced, inhibited, or abrogated expression or activity of the target transcript is observed compared to a control sample not treated with the ASO. In some embodiments, an ASO of the present disclosure reduces the abundance and/or translational activity of the target *KCNT1* transcript in a treated sample, e.g., by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to a control sample not exposed to the ASO. In some cases, the ASO reduces the level of the target transcript *in vivo* by said percentage, and administration of the ASO optionally results in a tolerability score (Functional Observational Battery or FOB score) of less than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1, e.g., 0. The terms “reduce” and “inhibit” do not necessarily mean a total elimination of the entire amount and/or activity of the transcript. In some embodiments, ASOs are considered to be active when they reduce the amount or activity of the target RNA by 25% or more in an *in vitro* assay. The present ASO may cause a detectable or measurable change in the level or activity of the *KCNT1* protein encoded by the target RNA.

Without wishing to be bound by theory, it is believed that ASOs may inhibit expression of *KCNT1* protein by recruiting an RNase H1 enzyme to the duplex formed between an ASO and the target *KCNT1* transcript. Enzymes of the RNase H1 family are endonucleases that typically target RNA:DNA duplexes and catalyze the hydrolytic cleavage of the RNA in the duplex.

In some embodiments, the ASO has minimal off-target effects, and does not hybridize to any non-*KCNT1* transcript in a way that results in significant reduction in the abundance or activity of the non-*KCNT1* transcript.

A. Lengths of Antisense Oligonucleotides

In some embodiments, the present ASOs are between 8 and 30 nucleotides in length (e.g., 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length). In some embodiments, an ASO described herein can comprise a sequence, complementary to a same-length *KCNT1* transcript sequence, that is any of a range of nucleotide lengths having an upper limit of 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 and an independently selected lower limit of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

In certain embodiments, the complementary sequence in the ASO is between 16 and 20 nucleotides in length. In particular embodiments, the complementary sequence in the ASO is 16, 17, 18, or 20 nucleobases in length.

B. Modifications of Antisense Oligonucleotides

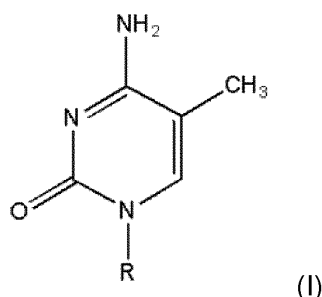
In some embodiments, the ASOs of the present disclosure may comprise one or more modifications, e.g., to increase binding affinity to the target transcript, increase ASO stability (e.g., increase resistance to degradation, e.g., by nucleases), and/or increase ease of ASO transport into the cell. Modifications may include any modification known in the art, including, for example, end modifications, nucleobase modifications, sugar modifications or replacements, and backbone modifications. End modifications may include, for example, 5' and/or 3' end modifications (e.g., phosphorylation, conjugation, DNA nucleotides, and inverted linkages). Base modifications may include, e.g., replacement with stabilizing bases, removal of bases, or conjugated bases. Sugar modifications or replacements may include, e.g., modifications at the 2' and/or 4' position of the ribose moiety, or replacement of the ribose moiety. Backbone modifications or internucleoside linkage modifications may include, for example, modification or replacement of phosphodiester linkages, e.g., with one or more phosphorothioates, phosphorodithioates, phosphotriesters, methyl and other alkyl phosphonates, phosphinates, and phosphoramidates.

In some embodiments, the present ASOs may have one or more modified nucleosides. The term "nucleoside" refers to a compound comprising a nucleobase and a sugar moiety. Naturally occurring nucleosides include DNA and RNA nucleosides. In a non-naturally occurring nucleoside (also referred to as a "modified nucleoside" or a "nucleoside analog"), the base and/or the sugar have been modified. The modification of the nucleoside may be "silent," in which case the modified nucleoside has the same or equivalent function in the context of the oligonucleotide compared to a naturally occurring nucleoside. In other cases, a modified nucleoside may increase the efficacy of the ASO in decreasing the abundance or activity of a target transcript. The term efficacy encompasses the target engagement on *KCNT1* mRNA.

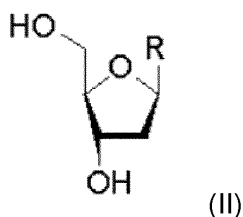
The term "nucleotide," as used herein, refers to a nucleoside covalently bonded to one or more modified or unmodified internucleoside linkages. Exemplary nucleotides include monophosphates, diphosphates, triphosphates, and thiophosphates. As used herein, the term "nucleotide" encompasses unmodified nucleotides (i.e., naturally occurring nucleotides) and modified nucleotides (i.e., nucleotide analogs). The term "nucleoside" encompasses unmodified nucleosides (i.e., naturally occurring nucleosides) and modified nucleosides (i.e., nucleoside

analogs); and the term “nucleobases” encompasses unmodified nucleobases (i.e., naturally occurring nucleobases) and modified nucleobases (i.e., nucleobase analogs).

In some embodiments, a modified nucleoside comprises a modified
5 nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine (5mC) nucleobase, as shown in the structure (I) below, wherein R represents the sugar moiety.

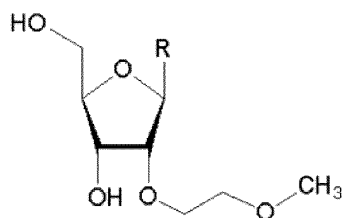


10 In some embodiments, a sugar moiety can be a modified or an unmodified sugar moiety. As used herein, an unmodified sugar moiety refers to a 2'-OH(H) ribosyl moiety as found in naturally occurring RNA, also referred to as an unmodified RNA sugar moiety. In some embodiments, a modified sugar moiety may be a 2'-H(H) deoxyribose sugar moiety. This moiety is found naturally in deoxyribonucleic acids
15 and may be referred to as an unmodified DNA sugar moiety or simply a DNA sugar moiety. The structure of a 2'-deoxynucleoside sugar moiety is shown in the structure (II) below, wherein R represents a nucleobase, and each of the 5'-hydroxyl and 3'-hydroxyl groups of the sugar is optionally involved in internucleoside linkages.



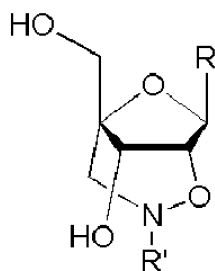
20 In some embodiments, a modified sugar moiety may comprise an O-methoxyethyl (MOE) moiety. In some embodiments, the O-methoxyethyl moiety is at the 2' position of the sugar, as shown in the structure below (III). R in the structure below represents a nucleobase. Each of the 5'-hydroxyl and 3'-hydroxyl groups of

the sugar is optionally involved in internucleoside linkages. A 2'-MOE modified sugar or 2'-MOE modified nucleoside, or simply an MOE sugar or nucleoside, is a ribose or nucleoside in which the 2' hydroxyl group that naturally occurs in the ribose is replaced with a 2'OCH₂CH₂OCH₃ group.



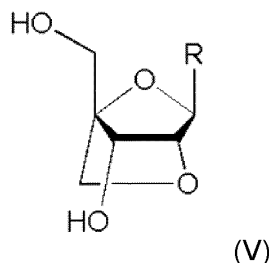
(III)

In some embodiments, a modified sugar moiety may comprise a bridged nucleic acid (BNA) moiety. A bridged nucleic acid comprises a bicyclic sugar moiety. The sugar moiety comprises a 4'-CH₂-NH-O-2' linkage. The nitrogen of the bridged nucleic acid is optionally substituted (e.g., methylated, alkylated, or modified with a phenyl group). The structure of a BNA moiety is shown below (IV), wherein R is a nucleobase, R' is, for example, an H, Me, or Phenyl group, and each of the 5'-hydroxyl and 3'-hydroxyl groups of the sugar is optionally involved in internucleoside linkages. In the present ASOs, R' is an Me group, unless otherwise specified. A BNA modified nucleoside, or simply a BNA nucleoside, is a nucleoside comprising a BNA sugar moiety.



In some embodiments, a modified sugar moiety may comprise a locked nucleic acid (LNA) moiety. A locked nucleic acid comprises a bicyclic sugar moiety. The sugar moiety comprises a 4'-CH₂-O-2' linkage. An LNA moiety, as described herein, may be in the alpha-L configuration or the beta-D configuration. In particular embodiments, LNA moieties in the ASOs described herein are in the beta-D configuration. The structure of an LNA moiety is shown below (V), wherein R is a nucleobase and each of the 5'-hydroxyl and 3'-hydroxyl groups of the sugar is

optionally involved in internucleoside linkages. An LNA modified nucleoside, or simply an LNA nucleoside, is a nucleoside comprising an LNA sugar moiety.



In certain embodiments, an ASO described herein may include one or more modified nucleotides known in the art, including, e.g., 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides, 2'-deoxy modified nucleotides, 2'-O-methoxyethyl modified nucleotides, modified nucleotides allowing for alternative internucleoside linkages (e.g., nucleotides comprising thiophosphates, phosphorothioates, and phosphotriesters), modified nucleotides terminally linked to a cholesterol derivative or lipophilic moiety, peptide nucleic acids, inverted deoxy or dideoxy modified nucleotides, abasic modifications of nucleotides, 2'-amino modified nucleotides, phosphoramidate modified nucleotides, modified nucleotides comprising modifications at other sites of the sugar or base of an oligonucleotide, and non-natural base-containing modified nucleotides.

In some embodiments, the ASO may include one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) modified nucleosides. In certain embodiments, all of the nucleosides in the ASO are modified nucleosides. In other embodiments, less than 100% of the nucleosides in the ASO (e.g., less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10%) are modified nucleosides.

The ASOs of the present disclosure may comprise naturally-occurring and/or non-naturally-occurring internucleoside linkages. The term "internucleoside linkage," as used in the present disclosure, refers to a covalent linkage between adjacent nucleosides in an oligonucleotide. In certain embodiments, an ASO described herein may include one or more modified nucleoside linkages known in the art, including, e.g., phosphate, phosphotriester, boranophosphate, methylphosphonate, phosphoramidate, phosphorothioate, phosphorodithioate linkage, methylenemethylimino (-CH₂-N(CH₃)-O-CH₂-), thiodiester, thionocarbamate (-O-C(=O)(NH)-S-), siloxane (-O-SiH₂-O-), dialkylsiloxane, N,N'-dimethylhydrazine (-CH₂-

N(CH₃)-N(CH₃)-, MMI (3'-CH₂-N(CH₃)-O-5'), amide-3 (3'-CH₂-C(=O)-N(H)-5'), amide-4 (3'-CH₂-N(H)-C(=O)-5'), amide-5 (3'-N(H)-C(=O)-CH₂-5'), amide-6 (3'-C(=O)-N(H)-CH₂-5'), formacetal (3'-O-CH₂-O-5'), methoxypropyl, thioformacetal (3'-S-CH₂-O-5'), carboxylate ester, carboxamide, sulfide, sulfonate ester, or amide
5 linker. See, for example: Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580; Chapters 3 and 4, 40-65.

In certain embodiments, an ASO described herein may include one or more modified nucleoside linkages known in the art, including, e.g., a phosphonoacetate
10 (PACE, P(CR'R'')_nCOOR) or thiophosphonoacetate (thioPACE, (S)-P(CR'R'')_nCOOR) internucleoside linkage, wherein n is an integer from 0 to 6 and each of R' and R'' is independently selected from the group consisting of H, an alkyl and substituted alkyl. Examples of these internucleoside linkages include phosphonocarboxylate, phosphonocarboxylate, thiophosphonocarboxylate, and
15 thiophosphonocarboxylate ester linkages, and in some embodiments are described in Yamada et al., *J. Am. Chem. Soc.* (2006) 128(15):5251-61, the contents of which are hereby incorporated by reference in its entirety.

In certain embodiments, the internucleoside linkage of a nucleotide may be a phosphate group or a thiophosphate group. Methods of preparation of phosphorous-
20 containing internucleoside linkages are well known to those skilled in the art. In particular embodiments, the ASOs described herein may have phosphodiester internucleoside linkages, phosphorothioate internucleoside linkages, or a combination thereof. The term "phosphodiester internucleoside linkage" refers to an internucleoside linkage between two nucleosides formed by a phosphodiester group.
25 The term "phosphorothioate internucleoside linkage" refers to a modified internucleoside linkage in which one of the non-bridging oxygen atoms of the phosphodiester internucleoside linkage is replaced with a sulfur atom.

In certain embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or can be prepared as separate enantiomers.
30 Representative internucleoside linkages having a chiral center include, but are not limited to, alkylphosphonates and phosphorothioates. ASOs of the present disclosure comprising internucleoside linkages having one or more chiral center(s) can be prepared as populations of ASOs comprising stereorandom internucleoside

linkages, or as populations of ASOs comprising stereodefined internucleoside linkages.

The term “stereodefined internucleoside linkage,” in the present disclosure, refers to an internucleoside linkage in which the stereochemical designation of the phosphorus atom is controlled such that a specific amount of R_p or S_p of the internucleoside linkage is present within an ASO strand. The stereochemical designation of a chiral linkage can be defined by, for example, asymmetric synthesis. An ASO having at least one stereodefined internucleoside linkage can be referred to as a stereodefined ASO.

In some embodiments, the present ASOs are fully stereodefined. The term “fully stereodefined ASO,” as used in the present disclosure, refers to an ASO sequence having a defined chiral center (R_p or S_p) in each internucleoside linkage in the ASO. The term “partially stereodefined ASO,” as used in the present disclosure, refers to an ASO sequence having a defined chiral center (R_p or S_p) in at least one internucleoside linkage, but not in all of the internucleoside linkages of the ASO. Therefore, a partially stereodefined ASO can include linkages that are achiral or non-stereodefined in addition to at least one stereodefined linkage.

In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising one or more particular phosphorothioate internucleoside linkages in a particular stereochemical configuration. In certain embodiments, the particular configuration of the particular phosphorothioate linkage is present in at least 65%, 70%, 80%, 90%, or 99% of the molecules in the population. Such chirally enriched populations of modified oligonucleotides can be generated using synthetic methods known in the art such as, for example, the methods described in Oka et al., *JACS* (2003) 125:8307, Wan et al., *Nuc. Acid. Res.* (2014) 42:13456, and PCT Patent Publication WO 2017/015555.

Unless otherwise indicated, chiral internucleoside linkages of modified oligonucleotides described herein can be stereorandom or in a particular stereochemical configuration.

C. Antisense Oligonucleotide Conjugates

The present disclosure also provides antisense oligonucleotide conjugates (ASO conjugates) comprising one or more ASOs described herein. The term “ASO conjugate,” in the present disclosure, refers to an oligomeric compound comprising an antisense oligonucleotide that is covalently linked to one or more non-nucleotide

moieties (conjugate moieties). Conjugation of an oligonucleotide to one or more conjugate moieties may improve the pharmacology or pharmacokinetic properties of the ASO. For example, the conjugate moiety may affect the activity, cellular distribution, cellular uptake, binding, absorption, tissue distribution, cellular distribution, charge, clearance, bioavailability, metabolism, excretion, permeability, and/or or stability of the ASO. In particular, the conjugate moiety may help target the ASO to a specific region in the central nervous system. In some embodiments of an ASO described herein, the conjugate moiety may be a carbohydrate, a peptide (e.g., a cell surface receptor ligand), and/or a lipid (e.g., phospholipid).

10 PCT Patent Publications WO 1993/07883 and WO 2013/033230 provide suitable conjugate moieties for use with the ASOs of the present disclosure. Certain conjugate groups and conjugate moieties have been described previously, for example, in the following references: thioether moiety, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. NY. Acad. Sci.* (1992) 660:306-309; Manoharan et al.,
15 *Bioorg. Med. Chem. Lett.* (1993) 3:2765-70), phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.* (1995) 36:3651-4; Shea et al., *Nucl. Acids Res.* (1990) 18:3777-83), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides* (1995) 14:969-73), or adamantane acetic acid, a
20 tocopherol group (Nishina et al., *Molecular Therapy Nucleic Acids* (2015) 4:e220; and Nishina et al., *Molecular Therapy* (2008) 16:734-40), or a GalNAc moiety (e.g., PCT Patent Publications WO 2014/076196, WO 2014/207232, and WO 2014/179620).

In certain embodiments, conjugation of an ASO of the present disclosure to a
25 lipophilic moiety may increase the delivery of the ASO to cells of the central nervous system. The term "lipophilic moiety," in the present disclosure, broadly refers to any compound or chemical moiety having an affinity for lipids. The lipophilic moiety may generally comprise a saturated or unsaturated hydrocarbon chain, which may be cyclic or acyclic. The hydrocarbon chain may comprise various substituents and/or
30 one or more heteroatoms, such as an oxygen or a nitrogen atom. In certain embodiments, the lipophilic moiety is a(n) aliphatic, cyclic, alicyclic, polycyclic, aromatic, or polyalicyclic compound. In certain embodiments, the lipophilic moiety is a steroid (e.g., sterol). Steroids include, without limitation, bile acids (e.g., cholic acid,

deoxycholic acid and dehydrocholic acid), cortisone, digoxigenin, testosterone, cholesterol, and cationic steroids, such as cortisone.

Certain lipophilic conjugate groups and conjugate moieties have been described previously, for example, in the following references: cholesterol moiety
5 (Letsinger et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6553-6), cholic acid moiety (Manoharan et al., *Bioorg. Med. Chem. Lett.* (1994) 4:1053-60), thiocholesterol moiety (Oberhauser et al., *Nucl. Acids Res.* (1992) 20:533-8), aliphatic chain, e.g., do-decan-diol or undecyl residues (Saison-Behmoaras et al., *EMBO J.* (1991) 10:1111-8; Kabanov et al., *FEBS Lett.* (1990) 259:327-30; Svinarchuk et al.,
10 *Biochimie* (1993) 75:49-54), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta* (1995) 1264:229-37), or an octadecylamine or hexylamino-5 carbonyl-oxycholesterol moiety (Crooke et al., *J Pharmacol. Exp. Ther.* (1996) 277:923-37).

D. Exemplary Antisense Oligonucleotide Compounds

Certain abbreviations are used in the present disclosure to describe the
15 modifications of each of the nucleotides and internucleoside linkages of ASOs described herein that are modified oligonucleotides. Abbreviations are as follows: A is an adenine nucleobase; G is a guanine nucleobase; T is a thymine nucleobase; C is a cytosine nucleobase, mC is a 5-methylcytosine nucleobase; e is a 2'-MOE modified sugar (e.g., a modified deoxyribose); d is a 2'-deoxyribose sugar; o is a
20 phosphodiester internucleoside linkage; and s is a phosphorothioate internucleoside linkage.

In certain embodiments, the ASOs of the present disclosure are gapmers. The term "gapmer," as used in the present disclosure, refers to an oligonucleotide comprising or consisting of an internal region positioned between two external
25 regions, wherein the sugar moieties of the nucleosides comprising the internal region are chemically distinct from the sugar moieties of the nucleosides comprising the external region. The term "gap" refers to the internal region of the oligonucleotide, while the term "wing" refers to the external regions. A gapmer has a 5'-wing, a gap, and a 3'-wing. The three regions form a contiguous sequence. The sugar moieties of
30 each of the wing nucleosides differ from at least some of the sugar moieties of the gap nucleosides. Unless otherwise noted, the nucleosides of the gap region of the ASOs of the present disclosure comprise entirely 2'-deoxyriboxyl nucleosides. In some embodiments, a gapmer may comprise one or more modified internucleoside

linkages and/or modified nucleobases that do not necessarily follow the gapmer pattern of sugar modifications.

In some embodiments, the oligonucleotides of the present disclosure are gapmers that comprise MOE, BNA, LNA, or DNA modifications, or any combination thereof. In some embodiments, the gapmers comprise MOE, DNA moieties. In certain embodiments, the internucleoside linkages between the oligonucleosides are phosphodiester or phosphorothioate internucleoside linkages, or a combination thereof.

The lengths of the three gapmer regions may be notated using the notation [# of nucleosides in the 5' wing]–[number of nucleosides in the gap]–[number of nucleosides in the 3' wing]. Thus, a 4-10-4 gapmer comprises 4 linked nucleosides in each wing and 10 linked nucleosides in the gap.

In some embodiments, an ASO of the present disclosure is a 3-10-3 LNA gapmer. 3-10-3 LNA gapmers are 16 nucleobases in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and each of the 5' and 3' wing segments comprises three LNA nucleosides. In some embodiments, all cytosine nucleobases throughout the 3-10-3 LNA gapmer are 5-methylcytosines. In some embodiments, all internucleoside linkages are phosphorothioate internucleoside linkages.

In some embodiments, an ASO of the present disclosure is a 3-11-3 LNA gapmer. 3-11-3 LNA gapmers are 17 nucleobases in length, wherein the central gap segment comprises 11 2'-deoxynucleosides and each of the 5' and 3' wing segments comprises three LNA nucleosides. In some embodiments, all cytosine nucleobases throughout the 3-11-3 LNA gapmer are 5-methylcytosines. In some embodiments, all internucleoside linkages are phosphorothioate internucleoside linkages.

In some embodiments, an ASO of the present disclosure is a 4-10-4 MOE gapmer. 4-10-4 gapmers are 18 nucleobases in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and each of the 5' and 3' wing segments comprises four 2'-MOE nucleosides. In some embodiments, all cytosine nucleobases throughout the 4-10-4 MOE gapmer are 5-methylcytosines. In some embodiments, all internucleoside linkages are phosphorothioate internucleoside linkages.

In some embodiments, an ASO of the present disclosure is a 5-10-5 MOE gapmer. 5-10-5 gapmers are 20 nucleobases in length, wherein the central gap

segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on both 5' and 3' end comprising five 2'-MOE nucleosides. In some embodiments, all cytosine nucleobases throughout the 5-10-5 MOE gapmer are 5-methylcytosines. In some embodiments, all internucleoside linkages are phosphorothioate internucleoside linkages.

In some embodiments, an ASO of the present disclosure is a 5-10-5 MOE gapmer. 5-10-5 gapmers are 20 nucleobases in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on both 5' and 3' end comprising five 2'-MOE nucleosides. In some embodiments, all internucleoside linkages are phosphorothioate internucleoside linkages.

In some embodiments, an ASO of the present disclosure is a 5-10-5 MOE gapmer. 5-10-5 gapmers are 20 nucleobases in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on both 5' and 3' end comprising five 2'-MOE nucleosides. In some embodiments, all cytosine nucleobases throughout the 5-10-5 MOE gapmer are 5-methylcytosines. In some embodiments, the internucleoside linkages between the nucleosides at positions 2 and 3, 3 and 4, 4 and 5, and 16 and 17, 17 and 18, 18 and 19 are phosphodiester internucleoside linkages. In some embodiments, the remainder of the internucleoside linkages are phosphorothioate internucleoside linkages.

In some embodiments, an ASO of the present disclosure is a 5-10-5 MOE gapmer. 5-10-5 gapmers are 20 nucleobases in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked by wing segments on both 5' and 3' end comprising five 2'-MOE nucleosides. In some embodiments, the internucleoside linkages between the nucleosides at positions 2 and 3, 3 and 4, 4 and 5, and 16 and 17, 17 and 18, 18 and 19 are phosphodiester internucleoside linkages. In some embodiments, the remainder of the internucleoside linkages are phosphorothioate internucleoside linkages.

In a particular embodiment, the present disclosure provides the ASOs listed in the following table and described in more detail below.

Table 1. Representative ASOs

Unmodified ASO Sequence	MOE gapmer and 5-methylcytosine compound number	MOE gapmer compound number
ATCCCAGGTTTACCCGATTC (SEQ ID NO: 4)	00849 (SEQ ID NO: 7)	00765 (SEQ ID NO: 10)
TCCCAGGTTTACCCGATTCA (SEQ ID NO: 5)	00815 (SEQ ID NO: 8)	00764 (SEQ ID NO: 11)
TATCCCAGGTTTACCCGATT (SEQ ID NO: 6)	00816 (SEQ ID NO: 9)	00769 (SEQ ID NO: 12)

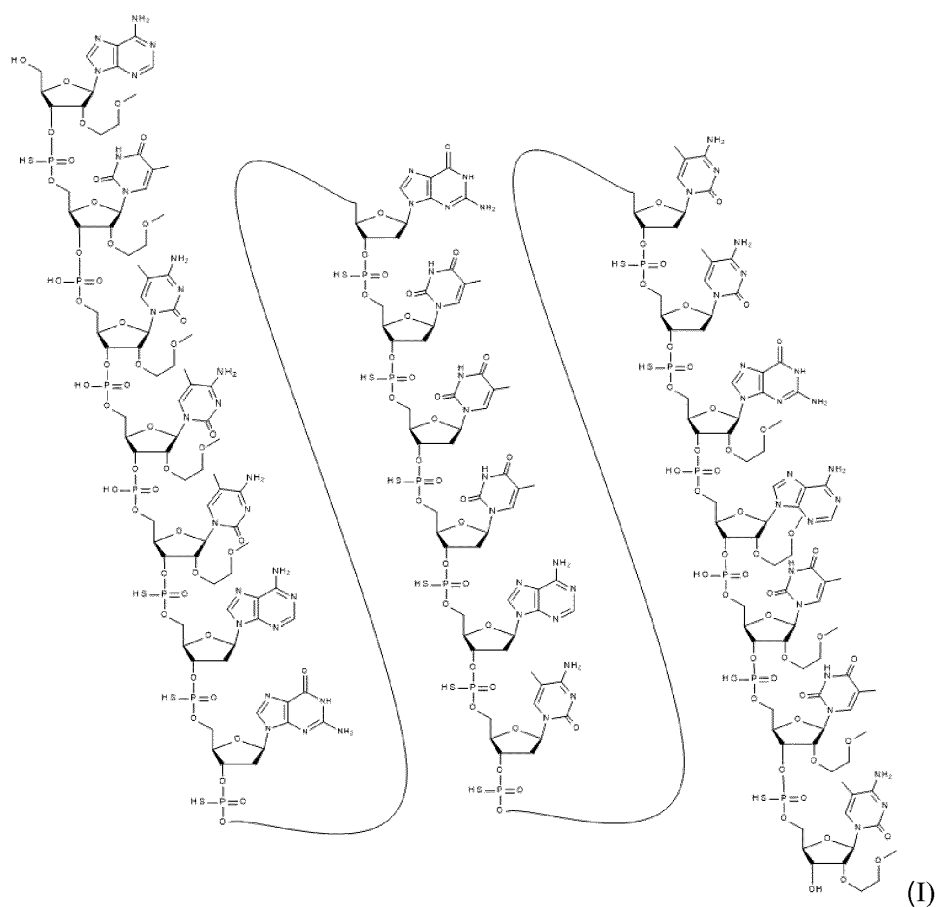
E. Representative MOE Gapmer Compounds

In some embodiments, an ASO of the present disclosure is a MOE gapmer compound, e.g., a compound described below.

Compound KCNT1_ASO_00849 is characterized as a 5MOE-10DNA-5MOE gapmer having a sequence, from 5' to 3', of ATCCCAGGTTTACCCGATTC (unmodified oligonucleotide SEQ ID NO: 4), wherein each of nucleosides 1-5 and 16-20 comprise a 2'-MOE modification, each of nucleosides 6-15 are 2'-deoxynucleosides, the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages, and each cytosine is a 5-methylcytosine.

Compound KCNT1_ASO_00849 is characterized by the following chemical notation: Aes Teo mCeo mCeo mCes Ads Gds Gds Tds Tds Tds Ads mCds mCds mCds Geo Aeο Teο Tes mCe (modified oligonucleotide SEQ ID NO: 7), wherein A is an adenine nucleobase, mC is a 5-methylcytosine nucleobase, G is a guanine nucleobase, T is a thymine nucleobase, e is a 2'-MOE modified sugar, d is a 2'-deoxyribose sugar, o is a phosphodiester internucleoside linkage, and s is a phosphorothioate internucleoside linkage.

Compound KCNT1_ASO_00849 is characterized by the following chemical structure (I):

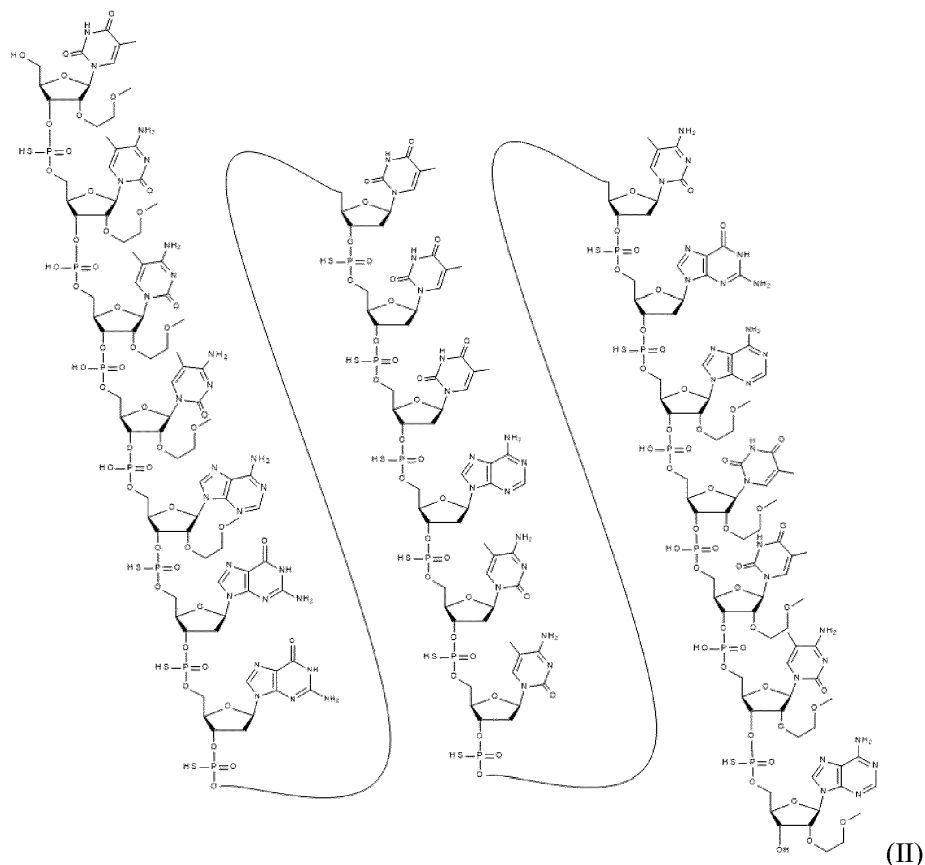


Compound KCNT1_ASO_00815 is characterized as a 5MOE-10DNA-5MOE gapmer having a sequence, from 5' to 3', of TCCCAGGTTTACCCGATTCA
 5 (unmodified oligonucleotide SEQ ID NO: 5), wherein each of nucleosides 1-5 and 16-20 comprise a 2'-MOE modification, each of nucleosides 6-15 are 2'-deoxynucleosides, the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages, and each cytosine is a 5-methylcytosine.
 10

Compound KCNT1_ASO_00815 is characterized by the following chemical notation: Tes mCeo mCeo mCeo Aes Gds Gds Tds Tds Tds Ads mCds mCds mCds
 Gds Aeo Teo Teo mCes Ae (modified oligonucleotide SEQ ID NO: 8), wherein A is
 an adenine nucleobase, mC is a 5-methylcytosine nucleobase, G is a guanine
 15 nucleobase, T is a thymine nucleobase, e is a 2'-MOE modified sugar, d is a 2'-

deoxyribose sugar, o is a phosphodiester internucleoside linkage, and s is a phosphorothioate internucleoside linkage.

Compound KCNT1_ASO_00815 is characterized by the following chemical structure (II):



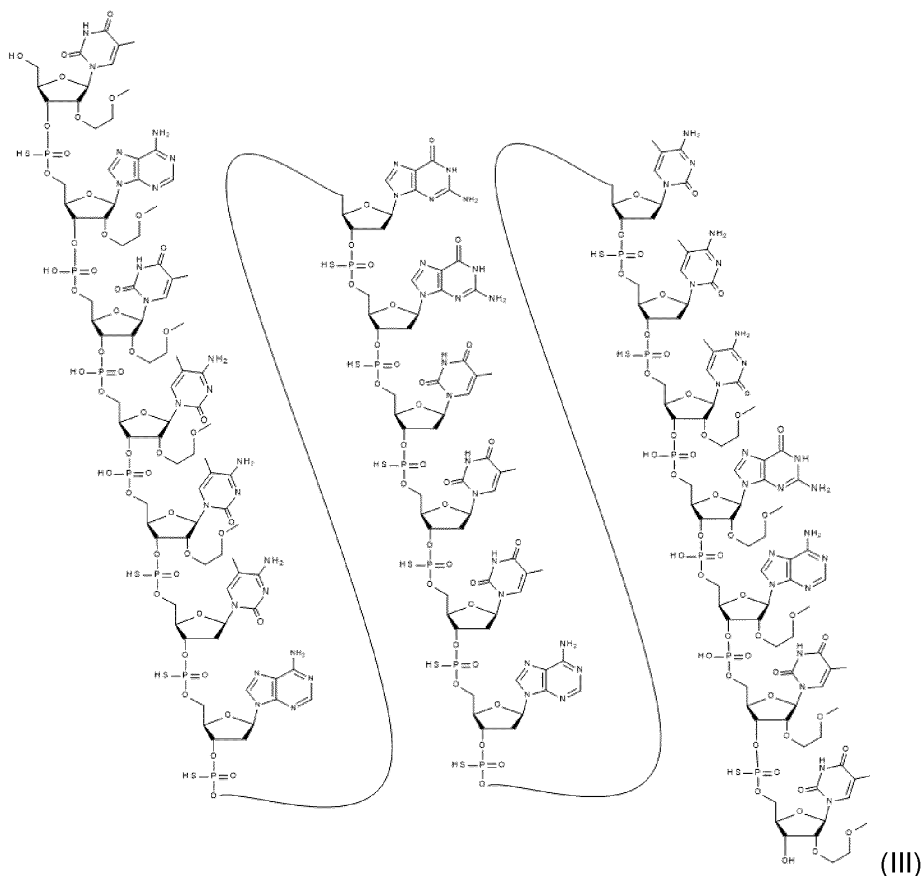
5

Compound KCNT1_ASO_00816 is characterized as a 5MOE-10DNA-5MOE gapmer having a sequence, from 5' to 3', of TATCCCAGGTTTACCCGATT (unmodified oligonucleotide SEQ ID NO: 6), wherein each of nucleosides 1-5 and 16-20 comprise a 2'-MOE modification, each of nucleosides 6-15 are 2'-deoxynucleosides, the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages, and each cytosine is a 5-methylcytosine.

Compound KCNT1_ASO_00816 is characterized by the following chemical notation: Tes Ae0 Teo mCeo mCes mCds Ads Gds Gds Tds Tds Tds Ads mCds

mCds mCeo Geo Aeo Tes Te (modified oligonucleotide SEQ ID NO: 9), wherein A is an adenine nucleobase, mC is a 5-methylcytosine nucleobase, G is a guanine nucleobase, T is a thymine nucleobase, e is a 2'-MOE modified sugar, d is a 2'-deoxyribose sugar, o is a phosphodiester internucleoside linkage, and s is a phosphorothioate internucleoside linkage.

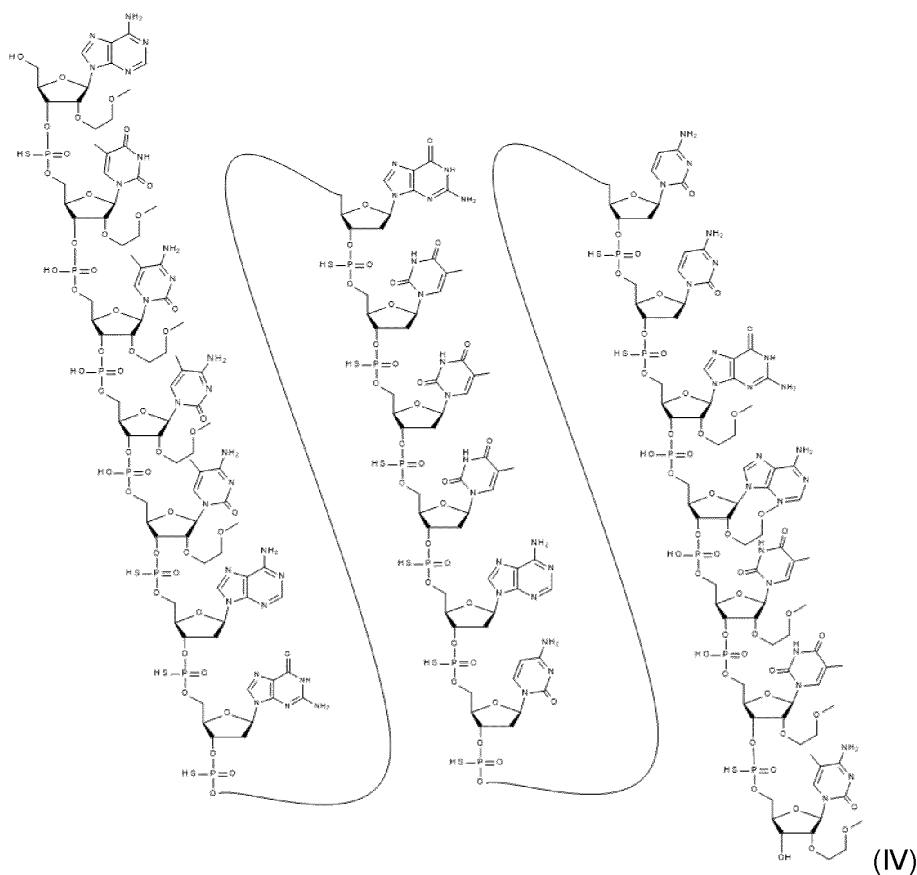
Compound KCNT1_ASO_00816 is characterized by the following chemical structure (III):



Compound KCNT1_ASO_00765 is characterized as a 5MOE-10DNA-5MOE gapmer having a sequence, from 5' to 3', of ATCCCAGGTTTACCCGATTC (unmodified oligonucleotide SEQ ID NO: 4), wherein each of nucleosides 1-5 and 16-20 comprise a 2'-MOE modification, each of nucleosides 6-15 are 2'-deoxynucleosides, the internucleoside linkages between nucleosides 1-2, 5-6 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages and cytosine with a 2'-MOE modification is a 5-methylcytosine.

Compound KCNT1_ASO_00765 is characterized by the following chemical notation: Aes Teo mCeo mCeo mCes Ads Gds Gds Tds Tds Tds Ads Cds Cds Cds Geo Aeo Teo Tes mCe (modified oligonucleotide SEQ ID NO: 10), wherein A is an adenine nucleobase, C is a cytosine nucleobase, mC is a 5-methylcytosine nucleobase, G is a guanine nucleobase, T is a thymine nucleobase, e is a 2'-MOE modified sugar, d is a 2'-deoxyribose sugar, o is a phosphodiester internucleoside linkage, and s is a phosphorothioate internucleoside linkage.

Compound KCNT1_ASO_00765 is characterized by the following chemical structure (IV):



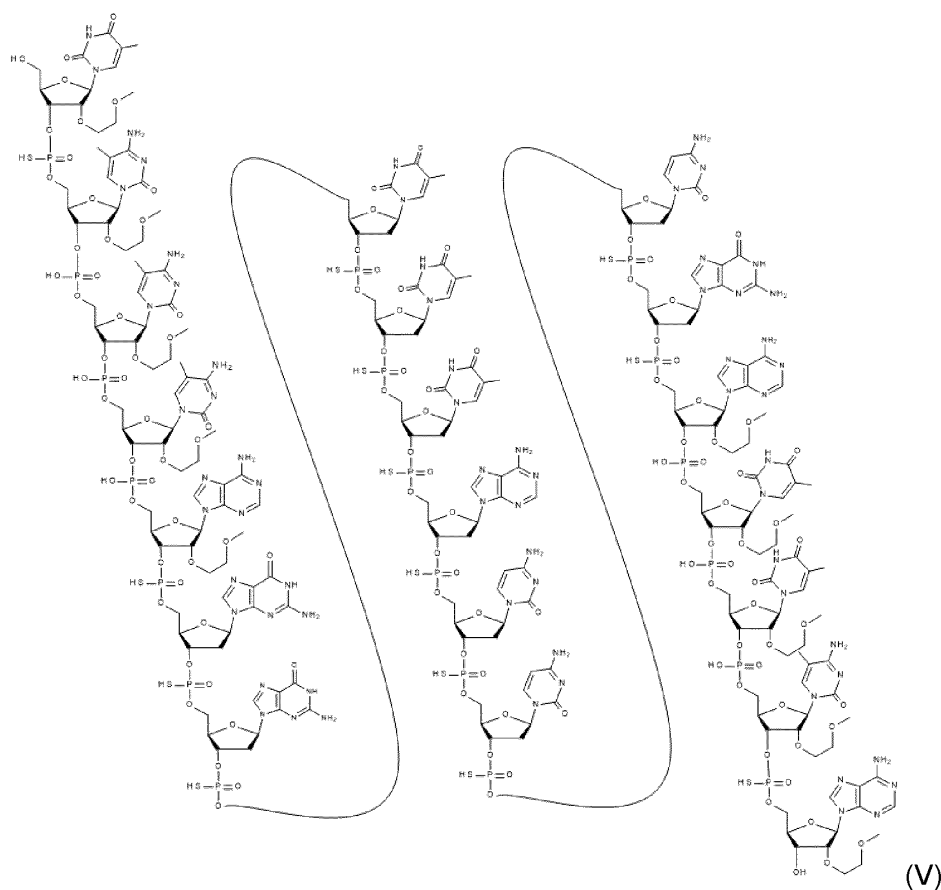
10

Compound KCNT1_ASO_00764 is characterized as a 5MOE-10DNA-5MOE gapmer having a sequence, from 5' to 3', of TCCCAGGTTTACCCGATTCA (unmodified oligonucleotide SEQ ID NO: 5), wherein each of nucleosides 1-5 and 16-20 comprise a 2'-MOE modification, each of nucleosides 6-15 are 2'-deoxynucleosides, the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside

linkages are phosphodiester internucleoside linkages and cytosine with a 2'-MOE modification is a 5-methylcytosine.

Compound KCNT1_ASO_00764 is characterized by the following chemical notation: Tes mCeo mCeo mCeo Aes Gds Gds Tds Tds Tds Ads Cds Cds Cds Gds
 5 AeO Teo Teo mCes Ae (modified oligonucleotide SEQ ID NO: 11), wherein A is an adenine nucleobase, C is a cytosine nucleobase, mC is a 5-methylcytosine nucleobase, G is a guanine nucleobase, T is a thymine nucleobase, e is a 2'-MOE modified sugar, d is a 2'-deoxyribose sugar, o is a phosphodiester internucleoside linkage, and s is a phosphorothioate internucleoside linkage.

10 Compound KCNT1_ASO_00764 is characterized by the following chemical structure (V):

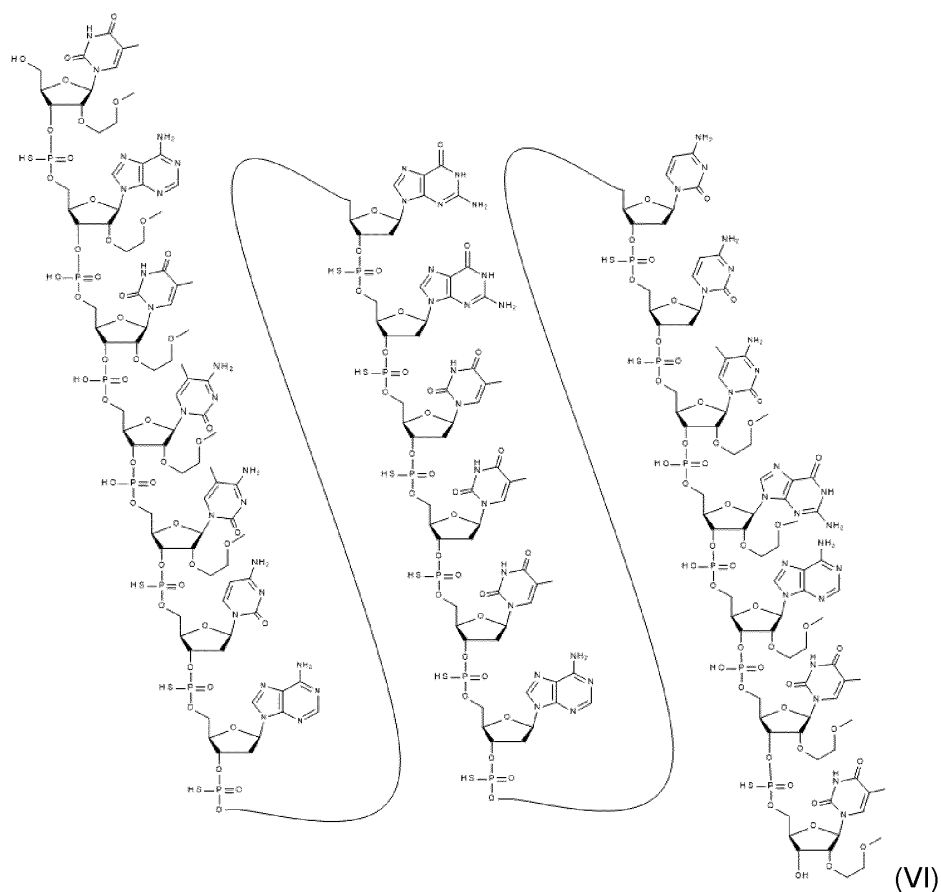


Compound KCNT1_ASO_00769 is characterized as a 5MOE-10DNA-5MOE
 15 gapmer having a sequence, from 5' to 3', of TATCCCAGGTTTACCCGATT (unmodified oligonucleotide SEQ ID NO: 6), wherein each of nucleosides 1-5 and

16-20 comprise a 2'-MOE modification, each of nucleosides 6-15 are 2'-deoxynucleosides, the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages and cytosine with a 2'-MOE
5 modification is a 5-methylcytosine.

Compound KCNT1_ASO_00769 is characterized by the following chemical notation: Tes Aeο Teο mCeο mCes Cds Ads Gds Gds Tds Tds Tds Ads Cds Cds mCeο Geo Aeο Tes Te (modified oligonucleotide SEQ ID NO: 12), wherein A is an adenine nucleobase, C is a cytosine nucleobase, mC is a 5-methylcytosine
10 nucleobase, G is a guanine nucleobase, T is a thymine nucleobase, e is a 2'-MOE modified sugar, d is a 2'-deoxyribose sugar, o is a phosphodiester internucleoside linkage, and s is a phosphorothioate internucleoside linkage.

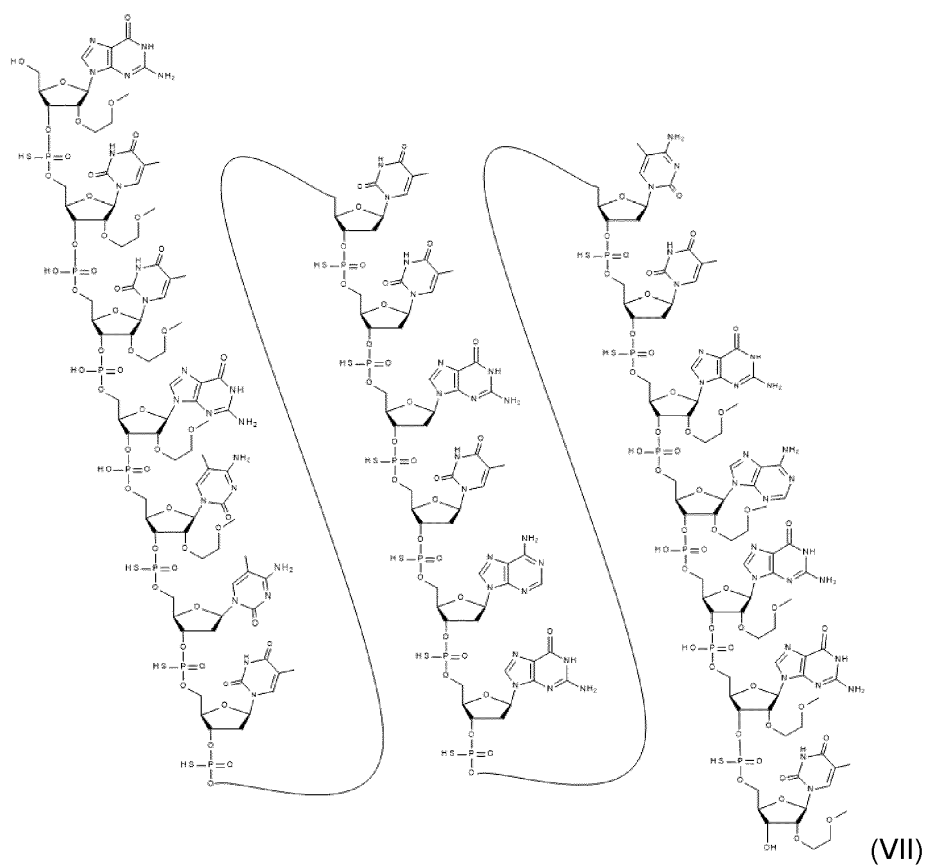
Compound KCNT1_ASO_00769 is characterized by the following chemical structure (VI):



Compound KCNT1_Valeriasen is characterized as a 5MOE-10DNA-5MOE gapmer having a sequence, from 5' to 3', of GTTGCCTTTGTAGCTGAGGT (unmodified oligonucleotide SEQ ID NO: 13), wherein each of nucleosides 1-5 and 16-20 comprise a 2'-MOE modification, each of nucleosides 6-15 are 2'-deoxynucleosides, the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages, and each cytosine is a 5-methylcytosine.

Compound KCNT1_Valeriasen is characterized by the following chemical notation: Ges Teo Teo Geo mCes mCds Tds Tds Tds Gds Tds Ads Gds mCds Tds Geo Aeo Geo Ges Te (modified oligonucleotide SEQ ID NO: 14), wherein A is an adenine nucleobase, mC is a 5-methylcytosine nucleobase, G is a guanine nucleobase, T is a thymine nucleobase, e is a 2'-MOE modified sugar, d is a 2'-deoxyribose sugar, o is a phosphodiester internucleoside linkage, and s is a phosphorothioate internucleoside linkage.

Compound KCNT1_Valeriasen is characterized by the following chemical structure (VII):



III. Methods of Making Antisense Oligonucleotides

5 An antisense oligonucleotide of the present disclosure may be synthesized by any method known in the art. For example, an ASO may be synthesized by *in vitro* transcription and purification (e.g., using commercially available *in vitro* RNA synthesis kits), by transcription and purification from cells (e.g., cells comprising an expression cassette/vector encoding the ASO), by use of an automated solid-phase synthesizer, and the like. In solid-phase oligonucleotide synthesis, monomeric nucleoside units are added iteratively to a growing oligonucleotide chain covalently bound to a solid support. In the case of phosphodiester linkages, electrophilic 3' phosphoramidite monomeric units may be used. However, any suitable electrophilic group can be used to covalently link two nucleosides.

15 The present ASOs may be purified following solid-phase synthesis through any method known in the art. For example, oligonucleotides may be precipitated from solution through treatment of the solution with ethanol and divalent cations. The

present ASOs may also be purified using, e.g., sizing columns, reverse-phase chromatography, high-performance liquid chromatography, and polyacrylamide gel electrophoresis.

Exemplary methods of synthesizing antisense oligonucleotides using solid-phase supports and purifying said oligonucleotides are described in, for example, Ellington et al., Introduction to the synthesis and purification of oligonucleotides. *Curr. Protoc. Nucleic Acid Chem.* (2001) Appendix 3C.

IV. Compositions of Antisense Oligonucleotides

10 In some embodiments, the present disclosure relates to compositions (e.g., pharmaceutical compositions) comprising an ASO described herein. In some embodiments, the composition is useful for treating a disease or disorder associated with expression or overexpression of KCNT1, e.g., DEE. Compositions of the present disclosure may be formulated based upon the mode of delivery.

15 A pharmaceutical composition described herein may comprise a pharmaceutically acceptable excipient. A pharmaceutically acceptable excipient can be liquid or solid and may be selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, and other pertinent transport and chemical properties. Any known pharmaceutically acceptable carrier or diluent
20 may be used, including, for example, water, saline solution, buffering agents, preservatives, and the like. For example, the ASOs of the present disclosure may be administered to a patient as a formulation in phosphate buffered saline (PBS). Example of pharmaceutically acceptable excipients include water, saline, buffer solution, or artificial cerebrospinal fluid. The pharmaceutically acceptable excipient is
25 preferably sterile.

The ASOs of the present disclosure may be administered as pharmaceutically acceptable salts. A pharmaceutically acceptable salt is a salt of the ASOs of the present disclosure that is physiologically acceptable and retains the desired biological activity of the ASO without having undesired toxicological effects. As used
30 herein, the term ASO encompasses both the free acid form and salt forms (e.g., sodium salt form) of the oligonucleotides.

The ASOs of the present disclosure may be admixed, encapsulated (e.g., in a lipid nanoparticles), conjugated, or otherwise associated with other molecules, molecular structures, or mixtures of nucleic acids.

U.S. Patent Publication Number 2020/0385723 provides suitable pharmaceutical compositions for use with the ASOs of the present disclosure.

V. Methods of Using Antisense Oligonucleotides

5 The ASOs of the present disclosure typically inhibit the activity of transcripts encoded by the *KCNT1* gene in a mammalian cell, such as a human cell. In some embodiments, the cell is a neuronal cell. In certain embodiments, the cell is a cell of the central nervous system (CNS), including cells of the motor cortex, frontal cortex, caudate, amygdala, pons, substantia nigra, putamen, cerebellar peduncle, corpus
10 collosum, dorsal cochlear nucleus (DCN), entorhinal cortex (Ent Cortex), hippocampus, insular cortex, medulla oblongata, central gray matter, pulvinar, occipital cortex, cerebral cortex, temporal cortex, globus pallidus, superior colliculi, and basal forebrain nuclei.

 The present disclosure provides methods of down-regulating the abundance
15 or activity of *KCNT1* gene transcripts in cells or in tissues comprising contacting the cells or tissues with an effective amount of one or more of the ASOs or compositions of the disclosure. The methods may be carried out *in vitro* or *in vivo*.

 In some embodiments, the ASOs of the present disclosure can be utilized for treatment or prophylaxis. The ASOs of the present disclosure can be used as
20 therapeutics in animals suspected of having a disease or disorder that can be treated by modulating the expression of the *KCNT1* gene transcript and/or *KCNT1* protein. The animal may also be prone to having the disease or disorder associated with the expression of the *KCNT1* gene and is not necessarily suspected of having the disease or disorder. The animal is treated by administering a therapeutically or
25 prophylactically effective amount of one or more of the ASO compounds or pharmaceutical compositions of the present disclosure. In some embodiments, the animal is a mammal. In some embodiments, the animal is a human.

 In some embodiments, the ASOs described herein may be used to treat *KCNT1*-associated diseases, in particular DEE including EIMFS and EOEE. In
30 general, a *KCNT1*-associated disease results in high seizure burden and experience seizures.

 In some embodiments, the ASOs of the present disclosure ameliorate the symptoms of a disease or disorder associated with the expression of the *KCNT1* gene. Amelioration may refer to a reduction in the severity or the frequency of

occurrence of a symptom, such as severity and frequency of seizures. Amelioration may also refer to a delay in the onset or progression of a symptom such as seizures.

A “therapeutically effective amount” of an ASO as disclosed herein is an amount sufficient to carry out a specifically stated purpose. Such an amount can be
5 determined empirically and in a routine manner, in relation to the stated purpose. Certain factors may influence the dosage and timing required to effectively treat a subject, including, but not limited to, severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and one or more other diseases being present. Moreover, treatment of a subject with a therapeutically
10 effective amount of a pharmaceutical composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the ASOs of the present disclosure may be made using conventional methodologies or on the basis of *in vivo* testing using appropriate animal models. A therapeutically effective amount may alleviate the symptoms of a disease.

15 In certain embodiments, the ASOs or pharmaceutical compositions of the present disclosure are prepared for injection (e.g., intravenous, subcutaneous, intramuscular, intrathecal (IT), intracerebroventricular (ICV), intracranial, and the like). In preferred embodiments, the pharmaceutical composition is injected intrathecally or intracranially to the subject.

20 In some embodiments, the ASOs of the present disclosure can be used for research purposes. For example, an ASO may be used to specifically inhibit the synthesis of the KCNT1 protein in cells and experimental animals. ASO-mediated inhibition of KCNT1 synthesis can be used to perform functional analyses of KCNT1 protein.

25

VI. Kits and Articles of Manufacture

The present disclosure also provides kits and articles of manufacture comprising an ASO described herein. Kits or articles of manufacture comprising an ASO of the present disclosure can be used to perform the methods described herein.
30 A kit or article of manufacture comprises at least one ASO in one or more containers.

In some embodiments, a kit or an article of manufacture described herein may be used for the treatment and/or prevention of a disease associated with the expression of the *KCNT1* gene. The kit or article of manufacture may further comprise a container and a label or package insert on or associated with the

container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic, and may hold a composition which is by itself or combined with another composition effective for treating or preventing the disease and may have a sterile access port.

- 5 The kit or article of manufacture may further comprise a package insert indicating that the compositions can be used to treat a particular disease. Alternatively, or additionally, the article of manufacture or kit may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose
10 solution. It may further include other materials desirable from a commercial and/or user standpoint, including other buffers, diluents, filters, needles, and syringes.

In some embodiments, the kits contain all of the components necessary and/or sufficient to perform a detection assay, including all controls, directions for performing assays, and any necessary software for analysis and presentation of
15 results. One skilled in the art will readily recognize that the disclosed ASO can be readily incorporated into one of the established kit formats which are well known in the art.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly
20 understood by those of ordinary skill in the art. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure. In case of conflict, the present specification, including definitions, will control. Generally, nomenclature used in connection with, and techniques of neurology,
25 medicine, medicinal and pharmaceutical chemistry, and cell biology described herein are those well-known and commonly used in the art. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall
30 include the singular. Throughout this specification and embodiments, the words "have" and "comprise" or variations such as "has" "having" "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. As used herein, the term "approximately" or "about" as applied to one or more values of

interest refers to a value that is similar to a stated reference value. In certain embodiments, the term refers to a range of values that fall within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context.

5 According to the present disclosure, back-references in the dependent claims are meant as short-hand writing for a direct and unambiguous disclosure of each and every combination of claims that is indicated by the back-reference. Further, headers herein are created for ease of organization and are not intended to limit the scope of the claimed invention in any manner.

10 All publications and other references mentioned herein are incorporated by reference in their entirety. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents form part of the common general knowledge in the art.

15 In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLES

20 The materials and methods used in the experiments described in the Examples below are as follows.

Construction of ASOs

25 The ASOs were designed to be complementary to the sense strand of the genomic *KCNT1* sequence (chromosome:GRCh38.9:135701585:135796108:1, SEQ ID NO: 3) as well as the sequence of the mRNA transcribed from the *KCNT1* gene (ENST00000371757.7, SEQ ID NO: 2) coding for the protein KCNT1-202 of 1235 amino acids and the sequence of the mRNA transcribed from the *KCNT1* gene (ENST00000628528.2, SEQ ID NO: 3) coding for the protein KCNT1-212 of 1211 amino acids.

Mice C57BL/6 Wildtype and Collection of Neurons

30 C57BL/6 wildtype mice weighed 20-30 g at their arrival and were housed in groups of three maximum per cage at room temperature with food and water available *ad libitum*. All procedures were approved by the Institut de Recherches SERVIER ethical committee in accordance with the principles of the Guide to the Care and Use of Experimental Animals.

Primary cortical neurons were collected from embryos removed from pregnant C57BL/6 wildtype mice at embryonic day 17.5. Cortical tissue of each embryo was dissected on ice-cold Hank's Balanced Salt Solution. Pooled tissue was minced and digested with papain at 37°C for 12 minutes. Digestion then was halted by the addition of 10% FBS/DMEM. The cells were triturated and resuspended in Neurobasal™ Plus medium supplemented with GlutaMAX™, 2% penicillin/streptomycin, and B-27™ Plus supplement. Cells were seeded at a density of 15000 cells/well onto 384-well poly-L-lysine + boric acid coated plates in 40 µL supplemented Neurobasal™ medium. The neurons were then incubated for four days at 37°C in a 5% CO₂ atmosphere.

ASO Screenings in Neurons in vitro

For single dose screenings, neurons were treated with 300 nM of the test ASO. For multiple dose screenings, neurons were treated with serial dilutions of the ASO starting at 3 µM (½ log dilutions, 11 concentrations total). Seven days after treatment with the ASO, the culture medium was replaced with new Neurobasal™ medium. 15 days after the treatment, RNA was extracted from the cells using the TaqMan™ Fast Advanced Cells-to-CT Kit (ThermoFisher). Cells were then washed in PBS and lysed in solution for five minutes at room temperature, with simultaneous DNase treatment. Lysis was terminated by treatment of the mixture with Stop Solution, followed by a two-minute incubation at room temperature. Reverse transcription was conducted immediately after cell lysis using Fast Advanced RT Enzyme Mix (ThermoFisher). The cDNA samples were then used for quantitative real-time PCR measurement using TaqMan™ genotyping assays. Specific probes and primers were used for *KCNT1* mRNA quantification (Mm01330661_g1 *KCNT1*; ThermoFisher). *KCNT1* mRNA levels were adjusted to the measured levels of the peptidylprolyl isomerase A housekeeping gene (*PPIA*) (probe/primer *PPIA_Mm02342430_g1*; ThermoFisher).

KCNT1 RNA expression was normalized to the expression of the *PPIA* housekeeping gene and *KCNT1* RNA expression was calculated using the $\Delta\Delta C_t$ ($\Delta\Delta C_t$) method. In a qPCR reaction, the quantification cycle value (C_t) is defined as the number of cycles required for the fluorescent signal to exceed the background fluorescence. The QuantStudio™ Real-Time PCR software program (Applied Biosystems, Foster City, CA) sets this threshold at ten standard deviations above the mean baseline fluorescence. The comparative C_t method normalized the C_t value of

KCNT1 target gene to *PPIA* housekeeping gene before comparisons are made between samples. First, the difference between Ct values (ΔCt) of the target gene and the housekeeping gene was calculated for each sample, and then the difference in the ΔCt ($\Delta\Delta\text{Ct}$) was calculated between two samples (e.g., control and treatment).

- 5 The fold-change in expression of the two samples was calculated as $2^{-\Delta\Delta\text{Ct}}$. The percentage of effect was calculated with this following formula $(2^{(-\Delta\Delta\text{Ct})}-1)*100$ in percent (%).

ASO efficacy in iPSC derived neurons

- 10 WT (Cellular Dynamics; C1012; lot 105286) and P924L (Cellular Dynamics; iPSC-NC; lot 01434.747.NC003) are human GABAergic neurons derived from induced pluripotent stem cells (iPSC; 01434). Immature neurons were seeded at 40000 cells/well in 100 μl of Complete Maintenance Medium composed of iCell Neural Base Medium (Cellular Dynamics; M1010; lot 105297) supplemented with iCell Neural Supplement A1 (Cellular Dynamics; M1032; lot 105480) in 96-well plates
- 15 coated with poly-ornithine (Sigma; P4957; lot RNBK2252) at 0.002% and laminin (Sigma; P2020; lot 132987) at 3.3 $\mu\text{g}/\text{mL}$ and incubated at 37°C, 5% CO_2 .

- The medium was completely replaced the next day with 100 μl of Complete Maintenance Medium, and 50% of medium was replaced every 2 days for 1 week. Neurons were treated twice at DIV-8, and DIV-15 with the ASO at 0.1 and 1 μM , and
- 20 50% of medium is replaced 5 days after treatment (DIV-13 and 20).

- After 2 weeks of treatment, neurons were washed in PBS, and RNA was isolated using the TaqMan Fast Advanced Cells-to-CT Kit (A35378; lots 1115521 and 1115536; ThermoFisher) following the manufacturer protocol. RNA was reverse transcribed immediately using Fast Advanced RT Enzyme Mix (4387410BF2; lot
- 25 ThermoFisher, lot 1091535). *KCNT1* transcripts were then measured by real time quantitative TaqMan™ PCR assays.

- Total *KCNT1* RNA expression was measured by real time quantitative PCR using TaqMan™ PCR assays with the probes Hs01063071_m1_KCNT1 (FAM) (lot P211111-008-H08; ThermoFisher). *PPIA* RNA was measured using the probes
- 30 *PPIA*-Hs99999904_m1 (VIC) (lot P211204-002-H10; ThermoFisher).

KCNT1 RNA expression was normalized to the expression of *PPIA* and *KCNT1* RNA expression was calculated using the ΔCt ($\Delta\Delta\text{Ct}$) method. In a qPCR reaction, the quantification cycle value (Ct) is defined as the number of cycles

required for the fluorescent signal to exceed the background fluorescence. The QuantStudio™ Real-Time PCR software program, sets this threshold at ten standard deviations above the mean baseline fluorescence. The comparative Ct method normalized the Ct value of *KCNT1* to *PPIA* before comparisons were made between samples. First, the difference between Ct values (ΔCt) of the target gene and the housekeeping gene was calculated for each sample, and then the difference in the ΔCt ($\Delta\Delta Ct$) was calculated between two samples (e.g., control and treatment). The fold-change in expression of the two samples was calculated as $2^{-\Delta\Delta Ct}$, and expressed as a percentage compared to the vehicle (%).

10 **Male Wistar Rats**

Male Wistar rats weighed 200-225 g at their arrival and were housed in groups of two per cage at room temperature with food and water available *ad libitum*. All procedures were approved by the Institut de Recherches SERVIER ethical committee in accordance with the principles of the Guide to the Care and Use of Experimental Animals.

ASO Solution Preparation

Sterile saline syringes and nuclease free centrifuge tubes were used to prepare dosing solutions. The tubes containing ASO powder were briefly centrifuged before adding saline solution, then re-centrifuged for 10 min to fully dissolve the ASO powder. The solution was vortexed for about 1 min and stored at 4°C until use.

Intracerebroventricular (ICV) Injection

C57BL/6 mice received a single unilateral bolus injection of the ASO at a dose of 30 nmol. Mice were anesthetized with 4.5-5% isoflurane and maintained during surgery with 1.5-2% isoflurane. For pain management, buprenorphine at a dosage of 0.04 mg/kg was administered subcutaneously at least 30 minutes before injection. The scalps of the mice were shaved and, following loss of the pedal reflex, mice were placed in a stereotaxic frame (David Kopf Instruments, CA). The scalp was sterilized using three alternating wipes of betadine and 70% ethanol. An incision was made in the scalp and the skull surface exposed and bregma positively identified. A hole was drilled in the skull at 0.5 mm AP, 1.1 mm ML, relative to bregma. The ASO was injected through a canula (31g) connected to a micro-syringe pump controller.

The dorsoventral DV coordinate was measured at 1 mm below the skull surface. Once the canula was positioned, the ASO solution was administered in 5 μ L of saline vehicle over 30 seconds. The canula was left in place for an additional three

minutes after injection to allow diffusion of the solution in the brain. After a slow withdrawing of the canula, the scalp was sutured and mice were subcutaneously injected with 1 mL of warm sterile saline solution to aid rehydration and placed in their warm home cage. A control group of mice was similarly dosed with saline vehicle control. Mice were observed until they regained consciousness and mobility to prevent potential adverse behavioral effects. Drug tolerability was scored one hour following dosing. Animals dosed with non-tolerated compounds (tolerability score >8) were euthanized immediately following the one-hour evaluation.

The ASOs described above were tested in C57BL/6 mice as described above to assess their tolerability profile. Valeriasen as described above was also tested as a comparator.

Intrathecal Injection

Male Wistar rats received a single intrathecal (IT) bolus injection of ASO at a dose of 2.5 mg. Rat were anesthetized with 4% isoflurane and maintained during surgery with 2-2.5% isoflurane. For pain management, carprofen at 5 mg/kg and buprenorphine at 0.05 mg/kg were administered subcutaneously at least 20 minutes before injection. Rat were shaved and following loss of the pedal reflex, an incision was made between the 5th and the 6th lumbar vertebra. Muscle around this area was dissected allowing to access of the spinal canal to insert the catheter used for ASO injection. Once the catheter was positioned, ASO solution was administered in 30µl of artificial cerebrospinal fluid (CSF) over ~30 seconds. The catheter was left in place and sealed to avoid diffusion of the CSF fluid. Muscle and skin were sutured, and the rats were subcutaneously injected with 1 mL warm sterile saline to aid rehydration and placed in their warm home cage. A control group of rats were similarly dosed with artificial CSF. Rats were observed until they regained consciousness and mobility to prevent potential adverse behavioral effects. Drug tolerability was scored at one, three, and 24 hours post dosing. Animals dosed with non-tolerated compounds (tolerability score >8) were euthanized immediately following the one-hour evaluation.

ASO Acute Tolerability Assessment

At one, three, and twenty-four hours post injection, adverse effects were monitored and scored in dosed rats according to the criteria shown in **FIG. 3**. A normal tolerability score is 0 and a highly toxic score corresponds to 14. The final tolerability score was calculated based on the sum of all criteria. If for some

oligonucleotides, an intolerable acute toxicity was observed without reaching the first observational time point, then the ASO was scored with an acute toxicity score of 14, and the mice were immediately euthanized. If a score of higher than 6 was measured at the one-hour time point, mice were more closely monitored over the course of the experiment.

ASO Long Term Tolerability Assessment

Mice were weighed on the injection day and three times per week until completion of the experiment. Any mice displaying intolerable health or behavioral observations, or weight loss of more than 20% of their initial body weight were immediately euthanized.

Tissue Sampling

All mice were euthanized by anesthetic overdose. Animals were transcardially perfused in the left ventricle with 0.9% saline. The thoracic aorta between the lungs and the liver was clamped with hemostatic forceps to block blood flow from the heart to the abdomen, but to allow blood to flow to the brain. The right ventricle was opened with scissors. A constant pressure of 100 to 120 mm Hg was maintained on the perfusion solution by connecting the solution bottle to a manometer-controlled air compressor. Perfusion was continued until the skull surface turned pale and perfusion solution exited the right ventricle. Following perfusion, brain tissues (cortex) were collected. Samples were cut into small pieces, mixed and aliquoted into three equal parts. All samples were frozen with liquid nitrogen and stored at -80°C until use for RNA, protein, and ASO measurements. For some studies, blood and CSF were also collected.

mRNA Measurement by qRT-PCR

RNA extraction and mRNA quantification by qPCR were performed. From right cortex biopsies, RNA was extracted using RNeasy Mini Kit (Qiagen) with DNase treatment. Total RNA samples were quantified using a Nanodrop™ spectrophotometer and analyzed using TapeStation to determine the quality of the RNA (RIN). In qPCR quantification experiments, the RNA was first reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™). The reaction was performed in a 100 µL final reaction volume, starting from 1000 ng of total RNA (to a final RNA concentration of 10 ng/µL). Quantification of human *KCNT1* mRNA and mouse *PPIA* mRNA were performed with 40 ng of total cDNA using QuantStudio™ 7 Flex (Applied Biosystems™),

TaqMan™ Universal PCR Master Mix (Applied Biosystems™, lot 4324020), and TaqMan™ Gene Expression Assays in duplex (Mm01330661_g1 in FAM fluorochrome and Mm02342430_g1 in VIC fluorochrome). qPCR analysis was performed in triplicate using the fast run mode. The Ct values of each qPCR plate were analyzed using Excel software. Technical replicates (n = 3) were combined and averaged to the geometric mean. Relative expression was generated for each ASO group using the mouse control PBS condition.

KCNT1 Protein Expression by Mass Spectrometry Analysis

Mouse brain tissues were homogenized with Precellys® (2x20s, 5000tr) in lysis buffer (ammonium bicarbonate 50mM, deoxycholate 1%, Sigma Protease and Phosphatase Inhibitor Cocktail, 1% deoxycholate) at 150 mg/mL. Brain homogenates were then centrifuged (27000g, 4°C, 20 minutes) and supernatants collected. Brain samples were heated at 95°C for five minutes. Next, trypsin (20 µg) was added into each sample. Tryptic digestion was performed by incubation at room temperature for 30 minutes followed by one minute in an ultrasonic bath (Branson 1200). The reaction was stopped by the addition of 1 µL of TCEP (0.5 M) and 1 µL of 100% formic acid followed by incubation at 95°C for five minutes. The samples were then centrifuged at 30000 g for 15 minutes. Peptide digests were analyzed by a reversed-phase liquid chromatography tandem mass spectrometry (LC-MS/MS) using a Shimadzu LC system (Shimadzu) coupled online to a triple quadrupole mass spectrometer (Shimadzu LCMS-8060) operated in the MRM mode. The specific peptide used to measure KCNT1 protein abundance were LFPSLSITTELTGPSNMR (SEQ ID NO: 15). The specific peptide used to measure GAPDH protein abundance was VGVNGFGR (SEQ ID NO: 16). A single 10 µL injection of each brain sample digest was injected on a Waters™ XBridge Peptide BEH C18 column (300 Å; 3.5 µm; 150 mm x 2.1 mm). Peptides were eluted using a linear gradient of acetonitrile (2-40%) in 0.1% formic acid over 30 min. Chromatograms were analyzed using the Shimadzu LabSolutions software. The signal intensity obtained for each peptide was normalized by GAPDH signal obtained in each sample and is expressed in arbitrary units (AU).

High Performance Liquid Chromatography (HPLC) Fluorescence

Samples were analyzed against a set of calibration standards prepared in water. As no matrix effect was noticed, quantification of all samples (plasma, CSF and tissues) were performed using a water set of standards. Frozen tissues were

weighed and grinded into MasterPure™/proteinase K 97/3 (V/V) buffer for 2x30 seconds at 6500 rpm using a Precellys device. Plasma samples (5µL) were diluted into MasterPure™/proteinase K 97/3 (70 µL). Plasma and tissue homogenates were incubated during 30 minutes at 55°C under soft agitation. Then, 10 µL of 3 M KCl solution was added into 50 µL of tissue homogenates or plasma dilution, rapidly vortexed and sonicated for five minutes. The tubes were centrifuged for ten minutes (20000 g) at 4°C. CSF samples (10 µL) were diluted into hybridization buffer (Tris HCl 50 mM pH 8.5 / ACN 90/10) (45 µL) and proteinase K (1 µL) and were incubated for 15 minutes at 55°C. Prior to analysis, a hybridization step was undertaken with a fluorescently labelled peptide nucleic acid oligomer complementary to the quantified oligonucleotide.

For calibration standards and tissue homogenates, 40 µL hybridization buffer was mixed with 10 µL of fluorescent complementary probe and 10 µL of calibration standards, quality control sample and study sample supernatants. For plasma samples, 30 µL hybridization buffer were mixed with 10 µL of fluorescent complementary probe and 60 µL of quality control sample and study sample supernatants. For CSF analysis, 10 µL of fluorescent complementary probe was directly added into the previous dilution. The mixtures were first incubated for 15 minutes at 95°C and then for 15 minutes at 55°C. Finally, samples were centrifuged for 5 minutes (20000 g) at 4°C. The samples were analyzed under an RP-HPLC system with fluorescence detection. The injected volume was 50 µL except for plasma (90µL). The amount of fluorescence due to hybridized oligonucleotide to fluorescent probe was measured and compared to the calibration curve. The oligonucleotide concentration in the sample was calculated considering the different dilutions used during sample preparation.

Example 1: mRNA Reduction *in vitro* Following a Single Dose of ASO

Modified oligonucleotides complementary to the human *KCNT1* genomic sequence were designed and tested *in vitro* in primary cortical neurons for their selective efficacy in reducing *KCNT1* mRNA levels. Neurons were treated with 300 nM of each ASO. mRNA levels were quantified using qRT-PCR (TaqMan™) as described above.

The modified oligonucleotides tested in this experiment are shown in **Table A** below. Each modified oligonucleotide listed in **Table A** is complementary to the

human *KCNT1* genomic sequence (SEQ ID NO: 3) and is a 5-10-5 MOE gapmer. The gapmers are 20 nucleobases in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and each of the wing segments comprises five 2'-MOE nucleosides. Cytosines are not 5-methylcytosines into the gap, and the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages.

Table A. 5-10-5 MOE Gapmers

Compound Name	% reduction (300 nM ASO)	Unmodified ASO Sequence	SEQ ID NO of Unmodified Oligonucleotide	Position in SEQ ID NO: 2
KCNT1_ASO_00764	89,80	TCCCAGGTTTACCCGATTCA	5	3475
KCNT1_ASO_00765	92.03	ATCCCAGGTTTACCCGATTG	4	3476
KCNT1_ASO_00769	89.00	TATCCCAGGTTTACCCGATT	6	3477

Example 2: mRNA Reduction *in vitro* Following Multiple Doses of MOE-modified ASOs

Modified oligonucleotides complementary to the human *KCNT1* genomic sequence were tested *in vitro* in primary cortical neurons for their selective efficacy in reducing *KCNT1* mRNA levels. Neurons were treated with multiple doses ($C_{max} = 3 \mu\text{M}$; $\frac{1}{2}$ log dilution; 11 concentrations) of the given ASO. This assay provided the *in vitro* cellular potency ($\text{pIC}_{50} = -\text{Log}(\text{IC}_{50})$) and efficacy (Delta Inhib Obs (%)) of the given ASO in neurons. In addition, the Hill coefficient, which is the slope of the line in a Hill plot, was measured in order to observe the shape of the dose response curve for each ASO.

Each of the ASOs in **Table B** is a 5-10-5 MOE gapmer. The gapmers are 20 nucleobases in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and each of the flanking wing segments comprises five 2'-MOE nucleosides. Cytosines are not 5-methylcytosines into the gap, and the internucleoside linkages between nucleosides 1-2, 5-16 and 19-20 are phosphorothioate internucleoside linkages, the other internucleoside linkages are phosphodiester internucleoside linkages.

FIG. 1 shows respective curves of inhibition of *KCNT1* mRNA expression in function of concentrations of KCNT1_ASO_00764, KCNT1_ASO_00765, and

KCNT1_ASO_00769 to measure IC₅₀. As multiple doses were tested, potency (pIC₅₀ = -LogIC₅₀), efficacy (Inhib Obs %) and Hill coefficient in primary neurons were calculated following Screener rules (Genedata software).

Table B. mRNA Reduction following multiple doses of MOE-Modified ASOs

Compound ID	Inhib Obs (%)	IC ₅₀ (M)	nH (-)	pIC ₅₀ (-)
KCNT1_ASO_00764	95.35	6.966E-10	0.5636	9.157
KCNT1_ASO_00765	100	2.429E-9	0.5036	8.615
KCNT1_ASO_00769	100	2.553E-7	0.6626	6.593

5

Example 3: mRNA Reduction *in vitro* in iPSCs

Cultured iPSC-derived neurons (WT or P924L mutant cells) were treated in free uptake condition with 100 nM or 1000 nM concentrations of modified ASOs. Neurons were treated twice at DIV-8, and DIV-15 with ASO at 0.1 and 1 µM, and 10 50% of medium was replaced 5 days after treatment (DIV-13 and 20). Modified oligonucleotides KCNT-1_ASO_00849 and KCNT1_Valeriasen are a 5-10-5 MOE gapmer with all cytosines residues throughout the gapmer being 5-methylcytosines.

Data in **Table C** are presented as the fold-change compared to the vehicle group (mean of six replicates). Statistical analyses were performed by the 15 Biostatistics Pre-Clinical Department using SAS v9.4. Significance thresholds were set to 5% for main effects. In order to evaluate treatment effect, a one-way anova with group factor was performed on the fold change (FC) of KCNT-1 expression followed by a Dunnett's test in order to compare treated groups versus vehicle group. The analysis was performed independently for WT and P924L mutated cells 20 data.

The data in **FIG.2** show that KCNT1_ASO_00849 has comparable potency to Valeriasen.

Table C. Mean mRNA Relative Expression in WT and Mutant P924L iPSC-Derived Neurons

25

Cells	Vehicle	KCNT1_ASO_00849 100 nM	KCNT1_ASO_00849 1000 nM	Valeriasen 100 nM	Valeriasen 1000 nM
WT	1.01	0.78	0.28	0.70	0.46
P924L	1.00	0.62	0.38	0.76	0.45

Example 4: Tolerability and Efficacy of Modified Oligonucleotides Complementary to Human *KCNT1* in C57BL/6 Wildtype Mice

4.1 Tolerability

Three-month-old C57BL/6 wildtype mice received a single bolus ICV injection of a modified oligonucleotide listed in **Table D** at a low dose of 10 nmol and a high dose of 60 nmol. Each modified oligonucleotide is complementary to the human *KCNT1* genomic sequence (SEQ ID NO: 3). The positions in the table indicate the 5' nucleoside to which the oligonucleotide is complementary in the *KCNT1*-212 transcript sequence (SEQ ID NO: 2).

For tolerability studies, the tolerability score is represented as the Functional Observational Battery (FOB) score at one-hour post-injection. Adverse effects were monitored and scored in dosed mice according to the criteria shown in **FIG.3**.

Table D. Tolerability at Low and High Doses in C57BL6/J Mice

Treatment	Unmodified Sequence	SEQ ID NO of Unmodified Oligo-nucleotide	Position in SEQ ID NO: 2	FOB (1h)
Dose tested 10 nmol				
PBS				0
KCNT1_ASO_00815	TCCCAGGTTTACCCGATTCA	5	3475	0
KCNT1_ASO_00816	TATCCCAGGTTTACCCGATT	6	3477	0
KCNT1_ASO_00849	ATCCCAGGTTTACCCGATTC	4	3476	0
KCNT1_Valeriasen	GTTGCCTTTGTAGCTGAGGT	13	517	0
Dose tested 60 nmol				
PBS				0
KCNT1_ASO_00815	TCCCAGGTTTACCCGATTCA	5	3475	4
KCNT1_ASO_00816	TATCCCAGGTTTACCCGATT	6	3477	0
KCNT1_ASO_00849	ATCCCAGGTTTACCCGATTC	4	3476	0.67
KCNT1_Valeriasen	GTTGCCTTTGTAGCTGAGGT	13	517	6

4.2 Efficacy

For efficacy studies, most treatment groups consisted of three animals. Mice were sacrificed two weeks after received a single bolus ICV injection of MOE modified oligonucleotides at a dose of 10 nmol or six weeks post-injection of a single bolus ICV injection of modified oligonucleotides at a dose of 60 nmol. Brain tissue was collected and the level of *KCNT1* mRNA was measured as described above.

Results are presented in **Table E** as percent reduction of the amount of *KCNT1* mRNA relative to vehicle control groups. A value of 0% reduction indicates that the compound had no effect.

The data in **FIG.4** show a better acute tolerability profile together with a comparable efficacy of KCNT1_ASO_815, KCNT1_ASO_849 to KCNT1_Valeriasen in mice after a single ICV administration.

Table E. Effects of Modified ASOs in C57BL6/J Mice at Low and High Doses

Treatment	Position in SEQ ID NO: 2	% <i>KCNT1</i> mRNA Reduction (Cortex)
ICV dose 10 nmol / time post-injection 2 weeks		
PBS		0
KCNT1_ASO_00815	3475	29
KCNT1_ASO_00816	3477	0
KCNT1_ASO_00849	3476	0
KCNT1_Valeriasen	517	7,5
ICV dose 60 nmol / time post-injection 6 weeks		
PBS		0
KCNT1_ASO_00815	3475	61.7
KCNT1_ASO_00849	3476	30.5
KCNT1_Valeriasen	517	30.5

4.3 Dose effect

Three-month-old C57BL6/J mice received a single bolus ICV injection of a modified oligonucleotide listed at doses as indicated. C57BL6/J mice were divided into groups of five. A group of four received PBS as a negative control for each experiment. For tolerability studies, the tolerability score is represented as the FOB score at one-hour post-injection.

For efficacy studies, mice were sacrificed three weeks post-injection. Cortical brain tissue was collected and the level of *KCNT1* mRNA was measured as described above by qRT-PCR. Results are presented in **Table F** as percent reduction of the amount of *KCNT1* mRNA relative to vehicle (PBS) control groups and normalized to mouse *PPIA*. A value of 0% reduction indicates that the compound had no effect.

The data in **FIG. 5** show a comparable dose response of KCNT1_ASO_815 and KCNT1_ASO_849 to KCNT1_Valeriasen in mice after a single ICV administration (multiple dose).

Table F. Dose Effect of Modified ASOs in C57BL6/J Mouse Cortex

Treatment	Dose (nmol)	FOB (1h)	% mRNA Reduction	ED50 (nmol)
KCNT1_ASO_00815	5	0	24.7	16
	15	0,2	48.7	
	30	2,20	64.2	
	60	5	75.5	
	100	8,2	80.5	
PBS		0	0	
KCNT1_ASO_00849	5	0	19.3	41
	15	0	29.4	
	30	0	42.2	
	60	1,2	57.5	
	100	4,2	60.4	
PBS		0	0	
KCNT1_Valeriasen	5	0	11.9	X
	15	1,4	14.8	
	30	7	22	
	60	14	X	
	100	14	X	
PBS		0	0	

4.4 Dose effect of ASO Concentration in the Cortex

In **Table G**, the amount of KCNT1_ASO_00849 and KCNT1_Valeriasen is
5 quantified per mg of cortex by HPLC fluorescence after a single ICV injection of
multiple doses. The data in Table G and in **FIG. 6** show that KCNT1_ASO_849 has
a better dose exposure relationship in mice cortex when compared to
KCNT1_Valeriasen at 5, 15 and 30 nmol doses.

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Table G. Dose Effect of ASO Concentration in the Cortex of C57BL6 Mice

Compound Name	Dose (nmol)	Cortex Median ASO concentration (fmol/mg)
KCNT1_ASO_00849	5	89.3
	15	240
	30	462
	60	711
	100	837
KCNT1_Valeriasen	5	45.6
	15	83
	30	102
	60	x
	100	x

Example 5: Duration of Action on Multiple Doses of Modified Oligonucleotides

Three-month old *C57BL6/J* mice were divided into groups of five to six mice. Each mouse received a single unilateral bolus injection of the test oligonucleotide at 20, 60 or 80 nmol as shown in **Table H** below. Two ASOs were tested in this experiment:

- KCNT1_ASO_00815:
modified ASO sequence: TCCCAGGTTTACCCGATTCA (SEQ ID NO: 8)
- KCNT1_ASO_00849:
modified ASO sequence: ATCCCAGGTTTACCCGATTC (SEQ ID NO: 7)

The animals were sacrificed at different timepoints (2, 6, 12, and 20 weeks) and RNA was extracted from cortex tissue and analyzed by qRT-PCR. A group of four mice received PBS as a negative control for each experiment. Results are presented as percent change of RNA levels relative to the PBS control and normalized to mouse peptidylprolyl isomerase A (PPIA).

The data in **FIG. 7** show that that both tested ASOs (KCNT1_ASO_00815 and KCNT1_ASO_00849) demonstrated dose-dependent inhibition of *KCNT1* mRNA expression in mouse cortex. The data also show that the inhibition was durable, lasting for at least 20 weeks. During the 20-week observation period, the *KCNT1* mRNA levels for both ASOs at both tested doses decreased continuously.

Table H. Inhibition of *KCNT1* mRNA in Mouse Cortex

Test Condition			Cortex <i>KCNT1</i> mRNA reduction (%)
Compound	Dose (nmol)	Timepoint (weeks)	
KCNT1_ASO_00815	20	2	51,5
		6	48,5
		12	33,4
		20	15,2
	60	2	67,8
		6	73,5
		12	57,1
		20	39,3
KCNT1_ASO_00849	20	2	36,9
		6	29
		12	15
		20	11,5
	80	2	58,9
		6	50
		12	34,6

		20	17,7
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Example 6: PK/PD in Mice

Three-month-old C57BL6/J mice received a single bolus intracerebroventricular (ICV) injection of a modified oligonucleotide listed at doses described in **Tables I and J**. C57BL6/J mice were divided into groups of five. A group of four mice received PBS as negative control for each experiment.

For tolerability studies, the tolerability score is represented as the Functional Observational Battery (FOB) score at one-hour post-injection. For efficacy studies, mice were sacrificed three weeks post-injection. Cortical brain tissue was collected and the level of *KCNT1* mRNA was measured as described above by qRT-PCR. Results are presented in the tables as percent reduction of the amount of *KCNT1* mRNA relative to vehicle (PBS) control groups and normalized to PPIA. A value of 0% reduction indicates that the compound had no effect. A dose response study was performed in mice to characterize the concentration effect relationship on mRNA expression level in target tissue.

The data in **FIG. 8** show that KCNT1_Valeriasen and KCNT1_ASO_00849 displayed similar dose-response inhibition of KCNT1 mRNA levels in the cortex.

Table I. Inhibition of KCNT1 mRNA Expression by KCNT1_Valeriasen

	KCNT1 mRNA reduction (%)	ASO concentration (fmol/mg)	KCNT1 mRNA reduction (%)	ASO concentration (fmol/mg)	KCNT1 mRNA reduction(%)	ASO concentration (fmol/mg)
nmol	5		15		30	
	87,00	74,56	83,00	116,94	79,00	172,01
	83,00	47,23	86,00	43,75	83,00	102,82
	93,00	29,44	95,00	82,79	74,00	85,97
	94,00	39,03	81,00	88,16	79,00	61,20
	87,00	37,87	84,00	83,27	77,00	87,61
Mean	88,80	45,62	86,00	82,98	78,40	101,92

Table J. Inhibition of KCNT1 mRNA Expression by KCNT1_ ASO_00849

	KCNT1 mRNA red. (%)	ASO conc. (fmol/mg)	KCNT1 mRNA red. (%)	ASO conc. (fmol/mg)	KCNT1 mRNA red. (%)	ASO conc. (fmol/mg)	KCNT1 mRNA red. (%)	ASO conc. (fmol/mg)	KCNT1 mRNA red. (%)	ASO conc. (fmol/mg)
nmol	5		15		30		60		100	
	81,00	102,86	69,00	344,57	59,00	475,51	45	646,00	39	872,27
	85,00	85,41	70,00	164,66	55,00	551,36	39	748,88	32	862,47
	81,00	124,29	70,00	208,40	44,00	600,00	45	629,26	40	826,97

	75,00	97,27	75,00	243,28	66,00	310,10	37	807,09	46	792,57
	84,00	36,69			66,00	371,15	47	722,83	42	828,27
Mean	81,20	89,30	71,00	240,23	58,00	461,62	42,60	710,81	39,80	836,51

Example 7: Tolerability of Modified Oligonucleotides Complementary to Human *KCNT1* in Rats

Male Wistar rats, divided into groups of four, received a single bolus IT
 5 injection of a modified oligonucleotide listed in **Table K** at a dose of 1 or 2.5 mg. Each modified oligonucleotide is complementary to the human *KCNT1* genomic nucleic acid sequence (SEQ ID NO: 3).

For tolerability studies, the tolerability score is represented as the Functional
 Observational Battery (FOB) score. The FOB was measured at one-hour post-
 10 injection, three hours post-injection, and twenty-four hours post-injection.

Table K. Long-Term Tolerability in Rats

Doses	1mg			2.5mg		
	FOB 1h (mean)	FOB 3h (mean)	FOB 24h (mean)	FOB 1h (mean)	FOB 3h (mean)	FOB 24h (mean)
KCNT1_Valeriasen	1.5	1.5	0	11	9	NA
KCNT1_ASO_00849	1.25	1	0	3	2.75	0.75
KCNT1_ASO_00815	2.4	1.6	0	3.2	3.6	0.2

Animals treated at a dose of 2.5 mg of KCNT1_Valeriasen had to be
 15 euthanized at approximatively 5 hours after dosing (premature kill) due to marked and severe clinical signs.

Example 8: *In vivo* brain Magnetic Resonance Imaging study

Twenty-four male Wistar rats weighing between 250 and 300 g, divided into
 20 three groups of eight animals, were anesthetized under isoflurane and placed on sterile operative field under optimal asepsis conditions to perform intrathecal (IT) injection. Animals received a single administration (80µL) of either artificial cerebrospinal fluid (aCSF), KCNT1_Valeriasen or KCNT1_ASO_815 by introducing a catheter at L5-L6 lumbar junction. The general study design was reviewed and
 25 approved by the Institut de Recherches SERVIER ethical committee, in general accordance with the animal health and welfare guidelines and standard operating procedures.

Brain MR images were performed using a 11.7-Tesla MR scanner system and ¹H-quadrature transmit-receive surface coil for rat head (Bruker). HR anatomical T2-weighted images were obtained using Multi Slices Multi Echoes (MSME) sequence (TE/TR: 5/7000 ms; in plane resolution: 160 x 160 μm²; slice thickness: 300 μm; slice number: n=70; total scan time: 14min). MRI acquisitions were performed for all animals prior treatment then two and four weeks after IT administration. Volumes of the lateral ventricles were assessed by manual delineation by the same experimenter on the brain anatomical images using π-PMOD (Biomedical image quantification software) as illustrated in **FIG. 9**.

The data in **FIG. 10** show that two weeks after administration, animals treated with KCNT1_Valeriasen displayed a significant 41.3% increase of total LV volume when compared to baseline. This abnormal enlargement was not observed anymore four weeks after IT injection, suggesting that the event was transient. No change in LV was observed in the animal groups treated with aCSF or KCNT1_ASO_815 two or four weeks after treatment.

SEQUENCES

The table below provides a list of SEQ ID NOs disclosed herein.

SEQ ID NO	Description
1	Human <i>KCNT1</i> cDNA encoding KCNT1-202
2	Human <i>KCNT1</i> cDNA encoding KCNT1-212
3	Human <i>KCNT1</i> gene
4	Unmodified sequence of ASO_765 and ASO_849
5	Unmodified sequence of ASO_764 and ASO_815
6	Unmodified sequence of ASO_769 and ASO_816
7	ASO_849
8	ASO_815
9	ASO_816
10	ASO_765
11	ASO_764
12	ASO_769

SEQ ID NO	Description
13	Unmodified sequence of ASO_Valeriasen
14	Valeriasen
15	Peptide
16	Peptide

CLAIMS

1. An antisense oligonucleotide for reducing KCNT1 expression, wherein the antisense oligonucleotide has a nucleobase sequence that comprises at least 12 consecutive nucleobases of any one of the nucleobase sequences of SEQ ID NOs: 4-12.
2. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide has a nucleobase sequence that comprises at least 15 consecutive nucleobases of any one of the nucleobase sequences of SEQ ID NOs: 4-12.
3. The antisense oligonucleotide of claim 1, wherein the antisense oligonucleotide has a nucleobase sequence of any one of SEQ ID NOs: 4-12.
4. The antisense oligonucleotide of claim 3, wherein the antisense oligonucleotide has a nucleobase sequence of any one of SEQ ID NOs: 7, 8 and 9.
5. The antisense oligonucleotide of any one of claims 1-4, wherein the antisense oligonucleotide has 18 to 20 linked nucleosides.
6. The antisense oligonucleotide of any one of claims 1-5, wherein at least one internucleoside linkage is a modified internucleoside linkage.
7. The antisense oligonucleotide of claim 6, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.
8. The antisense oligonucleotide of claim 7, wherein the phosphorothioate internucleoside linkages are preferably at positions 1-2, 5-16 and 19-20.
9. The antisense oligonucleotide of any one of claims 1-8, wherein at least one nucleoside of the antisense oligonucleotide comprises a modified sugar moiety.
10. The antisense oligonucleotide of claim 9, wherein the modified sugar moiety comprises a 2'-O-methoxyethyl group.

11. An oligonucleotide comprising the following formula:

i) Aes Teo mCeo mCeo mCes Ads Gds Gds Tds Tds Tds Ads mCds mCds mCds Geo AeO Teo Tes mCe (SEQ ID NO: 7);

ii) Tes mCeo mCeo mCeo Aes Gds Gds Tds Tds Tds Ads mCds mCds mCds Gds AeO Teo Teo mCes Ae (SEQ ID NO: 8);

iii) Tes AeO Teo mCeo mCes mCds Ads Gds Gds Tds Tds Tds Ads mCds mCds mCeo Geo AeO Tes Te (SEQ ID NO: 9);

iv) Aes Teo mCeo mCeo mCes Ads Gds Gds Tds Tds Tds Ads Cds Cds Cds Geo AeO Teo Tes mCe (SEQ ID NO: 10);

v) Tes mCeo mCeo mCeo Aes Gds Gds Tds Tds Tds Ads Cds Cds Cds Gds AeO Teo Teo mCes Ae (SEQ ID NO: 11);

vi) Tes AeO Teo mCeo mCes Cds Ads Gds Gds Tds Tds Tds Ads Cds Cds mCeo Geo AeO Tes Te (SEQ ID NO: 12)

wherein

A = an adenine

C = a cytosine

mC = a 5-methylcytosine

G = a guanine

T = a thymine

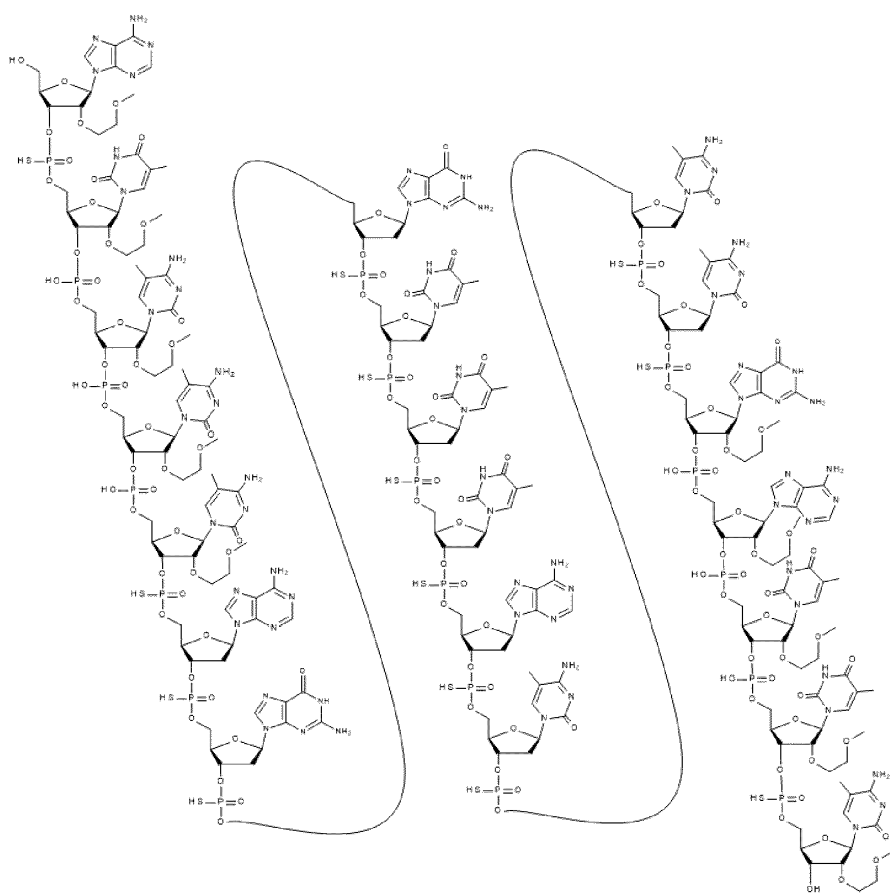
e = a 2'-O-methoxyethylribose

d = a 2'-deoxyribose

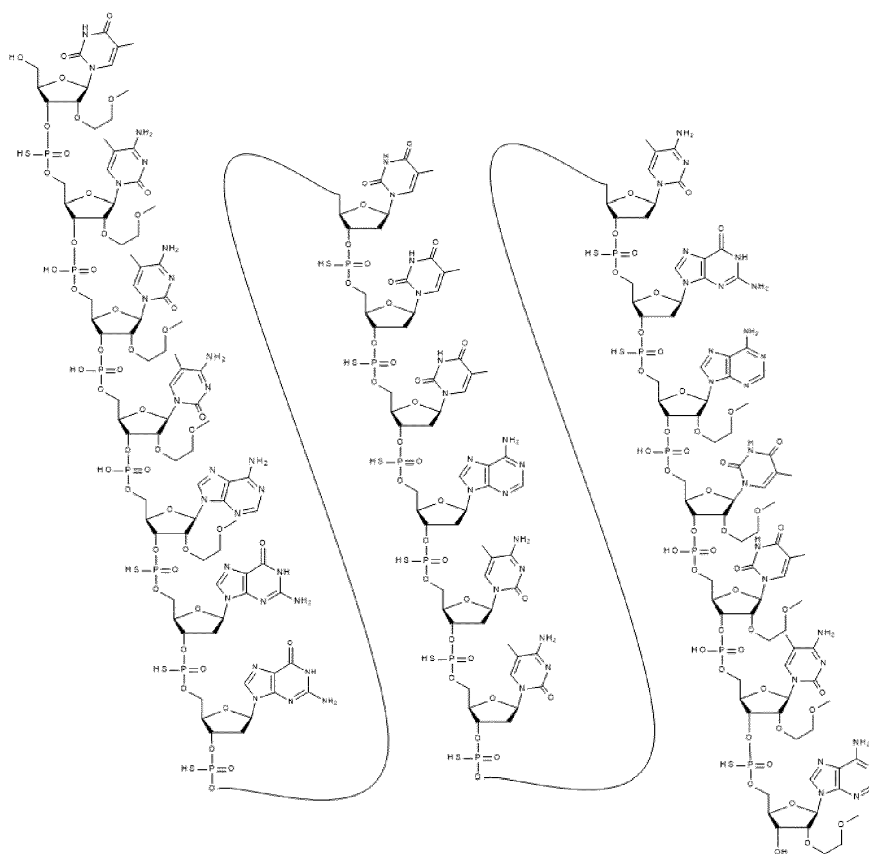
s = a phosphorothioate internucleoside linkage

o = a phosphodiester internucleoside linkage.

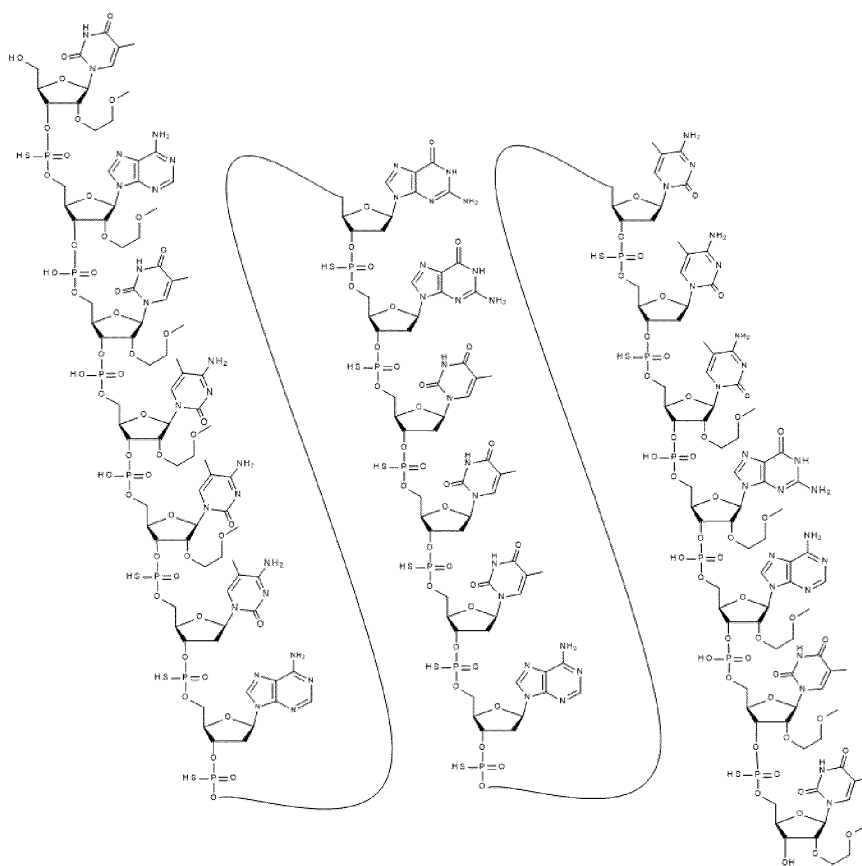
12. An oligonucleotide comprising the structural formula:



13. An oligonucleotide comprising the structural formula:



14. An oligonucleotide comprising the structural formula:



15. A conjugate comprising the antisense oligonucleotide according to any one of claims 1-14 and at least one conjugate moiety covalently attached to said oligonucleotide.

16. A pharmaceutical composition comprising the oligonucleotide of any one of claims 1-14 or the conjugate of claim 15 and a pharmaceutically acceptable excipient.

17. A method of reducing KCNT1 expression in a mammalian cell, comprising contacting the cell with the antisense oligonucleotide of any one of claims 1-14, the conjugate of claim 15, or the pharmaceutical composition of claim 16, thereby reducing KCNT1 expression in the cell.

18. The method of claim 17, wherein the cell is a cell in the central nervous system, optionally a cell in the human brain.

19. A method for treating developmental and epileptic encephalopathy (DEE) in a subject, optionally a human subject, in need thereof, comprising administering to the subject a therapeutically effective amount of the antisense oligonucleotide of any one of claims 1-14, the conjugate of claim 15, or the pharmaceutical composition of claim 16.

20. The method of 19, wherein the oligonucleotide is injected intrathecally or intracranially to the subject.

21. Use of the antisense oligonucleotide of any one of claims 1-14 or the conjugate of claim 15 for the manufacture of a medicament in treating DEE in a subject in need thereof in the method of any one of claims 17-20.

22. The oligonucleotide of any one of claims 1-14, the conjugate of claim 15, or the pharmaceutical composition of claim 16 for use in treating DEE in a subject in need thereof in the method of any one of claims 17-20.

23. The method, use, oligonucleotide for use, conjugate for use, or pharmaceutical composition for use of any one of claims 17-22, wherein the DEE is epilepsy of infancy with migrating focal seizures (EIMFS) or early onset epileptic encephalopathy (EOEE).